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(54) Title: ANTI-EGFR SINGLE-CHAIN FVS AND ANTI-EGFR ANTIBODIES			
(57) Abstract <p>This invention relates to new anti-EGFR antibodies and single-chain Fvs (scFvs) thereof which can be obtained from phage-antibody libraries constructed from cells of an immunized mammalian, preferably a mouse. Two of the single-chain Fvs isolated from the phage-antibody libraries were engineered to create partially humanized whole antibody molecules. These chimeric anti-EGFR antibodies contain constant regions of human immunoglobulins, and can be used as well as the single-chain Fvs as agents for the diagnosis and therapy of human tumors.</p>			

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Anti-EGFR Single-Chain Fvs and Anti-EGFR Antibodies

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TECHNICAL FIELD OF THE INVENTION

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This invention relates to new anti-EGFR antibodies and antibody fragments, preferably single-chain Fvs (scFvs) which can be obtained from phage-antibody libraries constructed from cells of an immunized mammalian, preferably a mouse. The antibody fragments isolated from the phage-antibody libraries can be engineered to create partially humanized whole antibody molecules. These chimeric anti-EGFR antibodies contain constant regions of human immunoglobulins, and can be used as well as their fragments as agents for the diagnosis and therapy of human tumors.

25

Furthermore, the invention demonstrates that phage-antibody libraries are an alternative, and more versatile, method for isolating antibodies from immunized mammals in comparison with the standard hybridoma technology.

30

The invention relates, moreover, to pharmaceutical compositions comprising said antibodies or fragments for the purposes of treating tumors like melanoma, glioma or carcinoma. The said antibodies or fragments can be used also for diagnostic applications regarding locating and assessing the said tumors in vitro or in vivo.

The specification relates to several technical terms which are herewith defined as follows:

"FRs" (framework regions) mean the four subregions of the light or
5 heavy chain variable regions that support the three CDRs.

"CDRs" (complementarity determining regions) mean the three subregions of the light or heavy chain variable regions which have hypervariable sequences and form loop structures that are primarily
10 responsible for making direct contact with antigen.

"Chimeric" or partially humanized antibodies mean antibodies comprising constant regions deriving from human sources and variable regions (CDRs included) deriving from non-human sources, e.g. from
15 the mouse.

"Humanized" or fully humanized antibodies mean antibodies comprising constant regions and FRs deriving from human sources whereas the CDRs derive from non-human sources.
20

"EGF" and "EGFR" mean the epidermal growth factor and its receptor.

"PCR" means the polymerase chain reaction.

"scFv" means single-chain Fv which is an antibody fragment.

25 "V_L" means light chain variable region.

"V_k" means kappa light chain variable region.

"V_H" means heavy chain variable region.

30 PBS means phosphate buffered saline

FCS means fetal calf serum

HBSS means Hanks balanced salt solution

FITC means fluoresceineisothiocyanate

35 MTC means mixed cell culture

BACKGROUND OF THE INVENTION

Epidermal growth factor (EGF) is a polypeptide hormone which is mitogenic for epidermal and epithelial cells. When EGF interacts with sensitive cells, it binds to membrane receptors (EGFR). The EGFR is a transmembrane glycoprotein of about 170 kD and is a gene product of the c-erb-B proto-oncogene.

10

MAb 425 is a murine monoclonal antibody raised against the well known human A431 carcinoma cell line (ATCC CRL 1555), binds to a polypeptide epitope of the external domain of the human EGFR, and inhibits the binding of EGF. MAb 425 (ATCC HB 9629) was found to mediate tumor cytotoxicity in vitro and to suppress tumor cell growth of epidermoid and colorectal carcinoma-derived cell lines in vitro (Rodeck et al., *Cancer Res.* 1987. 47: 3692). Humanized and chimeric versions of MAb 425 have been disclosed in WO 92/15683.

Over the last few years, methods have been described (Skerra and Plückthun, *Science* 1988. 240: 1038; Better et al., *Science* 1988. 240: 1041) with which functional antibody fragments can be produced in eukaryotic host cells, such as *E. coli*. These include the Fv fragment and the Fab fragment, whereby the Fv fragment is of special interest. Single-chain Fvs (wherein the V_L and the V_H chain are linked together) have been also described (Bird et al., *Science* 1988. 242: 423; Huston et al., *Proc. Natl. Acad. Sci. USA* 1988. 85: 5879).

Phage-antibody libraries offer an alternative technology to hybridoma technology in the isolation of antibodies from immunized animals. Hybridoma technology works by immortalizing the cells that produce

the antibodies. Phage-antibody technology works by immortalizing the genes that code for the antibodies (Winter, G. and Milstein, C., *Nature* 1991, 349: 293). In phage-antibody technology, the antibody heavy chain variable region (V_H) and light chain variable region (V_L) genes are PCR-amplified, the variable regions are randomly combined and expressed as antibody fragments on the surface of phage particles, and libraries of phage antibodies are screened for antibodies that bind to antigens of interest.

10

Hybridoma technology has been very successful at isolating mouse monoclonal antibodies when it has been possible to generate a strong immune response in the spleens of the animals. For example, mouse MAbs against human epidermal growth factor receptor (EGFR) have been isolated from the spleens of mice immunized intraperitoneally with human A431 tumor cells (Murthy et al., *Arch. Biochem. Biophys.* 1987, 252: 549). The potential advantage of phage-antibody technology over hybridoma technology is that virtually any source of antibody-expressing cells can be used as starting material and that large numbers of different antibodies can be rapidly screened. Another advantage of the phage-antibody technology is that the genes coding for the variable regions of the antibodies of interest have already been cloned and are immediately available for further genetic engineering. In one report, an anti-tetanus toxoid Fab fragment isolated from a phage-antibody library was converted into a whole antibody molecule (Bender et al., *Hum. Antibod. Hybridomas* 1993, 4: 74). During the last ten years, *in vitro* immunization has been used as an alternative technique to active immunization to generate monoclonal antibodies (mAbs) against a wide variety of antigens from both human

35

and murine systems (e.g. Vaux, D.J.T.; Helenius, A. and Mellman, I.; Nature, 1988, 336: 36; Gathuru, J.K et al.; J. Immunol. Methods, 1991, 137: 95; Borrebaeck, C.A.K.; Immunol. Today, 1988, 9:355). Advantages of this approach are that only small amounts of antigen are required and that the method is applicable for generating human hybridomas.

5 However, the generation of poor affinity IgM antibodies and the difficulty of immortalizing human lymphocytes after *in vitro* immunization have become persistent problems associated with this technology.

10 A new way of obtaining antibodies is by PCR amplification of repertoires of heavy (V_H) and light (V_L) chain variable region genes which are then randomly recombined and expressed as phage display libraries (7-9).

15 Antibody variable-region genes were cloned and fused to the minor coat protein (gene 3) as a single chain Fv fragment (scFv) (10). The phage particle displays on its surface the antibody fragment and can be selected by panning using the antibody's binding properties. This technology has

20 the advantage that random recombination of V genes may produce novel pairings with new specificities and affinities which could not be selected by natural processes. Moreover, such an approach makes possible the use of naive or *in vitro* immunized lymphocytes from murine or human sources.

25 Previous attempts to obtain mAbs against EGFR by murine B cells *in vitro* immunization and hybridoma technology rendered low-affinity, cross-reacting antibodies. In order to overcome such handicaps, the

30 combination of *in vitro* immunization followed by PCR cloning technology was carried out.

35 Therefore, it is an object of the invention to develop antibodies and antibody fragments which have a high affinity to the EGF-receptor and

which can be obtained by the advantageous procedure described above and below.

5

SUMMARY OF THE INVENTION

10 This invention compares mouse anti-EGFR antibodies isolated from three different phage-antibody libraries with a mouse MAb (425) isolated by standard hybridoma technology (Murthy et al., *Arch. Biochem. Biophys.* 1987. 252: 549; Kettleborough et al., *Protein Eng.* 1991. 4: 773). Libraries were prepared, not only from the spleen of an immunized mouse, but also from the draining lymph node of an immunized mouse and from in vitro immunized mouse cells. Two of the single-chain Fvs (scFvs) that were isolated from the libraries were 20 engineered to create chimeric whole antibody molecules with the mouse variable regions joined to human constant regions.

25 In detail, the present invention relates to an anti-EGFR single-chain Fv obtainable from phage-antibody libraries constructed from cells, preferably of the spleen or the draining lymph node of an immunized mammalian, preferably a mouse, or from in vitro immunized cells. In principle, the invention is not restricted to scFvs but extends also to 30 other anti-EGFR antibody fragments such as Fab or F(ab')₂.

35 Some of the scFvs according to the invention have well-defined DNA and amino acid sequences. Therefore, it is another object of the invention to provide a single-chain Fv fragment, wherein the variable

regions of the heavy and light chain comprise a DNA and/or a amino acid sequence selected from one of the heavy and light chain sequences given in Sequence Id. Nos. 1 - 32, preferably in Fig. 5 - 8.

5

Because in many cases only completely functioning, whole antibodies can be used for diagnostic and therapeutic purposes it is in the interest of the invention to link the variable regions from single-chain Fvs with
10 the constant regions of human immunoglobulins forming whole, partially or humanized anti-EGFR antibodies.

Therefore, it is an object of this invention to provide a whole anti-EGFR antibody constructed from DNA sequences deriving from antibody
15 fragments as defined above, below or as defined in the claims, and from DNA sequences deriving from constant regions of human immunogobulins, wherein, as a preferred embodiment, the heavy chain comprises the amino acid sequence of a gamma-1 chain, and the light
20 chain comprises the amino acid sequence of a kappa chain.

According to the present invention the anti-EGFR scFvs, are isolated by using the phage-antibody library technology. Therefore the invention relates to a process for the preparation of an anti-EGFR
25 single-chain Fv comprising the following steps:

- (i) isolating RNA from immunized mammalian cells, preferably mouse cells,
- 30 (ii) synthesizing first-strand cDNA,
- (iii) amplifying the V_H and V_k genes in cDNAs from the immunized cells,
- (iv) cloning said genes together with suitable restriction sites into a phagemid vector,
- 35 (v) transforming prokaryotic cells with the ligation mixtures,

- (vi) screening the phage libraries for phage antibodies directed to EGFR using purified EGFR, and
5 (vii) producing the desired single-chain Fv in prokaryotic host cells, preferably *E. coli*.

10 Additionally, it is an object of the invention to disclose a process for the preparation of a whole anti-EGFR antibody by cloning the DNA coding for the variable regions of anti-EGFR antibody fragments produced as indicated above or as defined in the claims into at least one eukaryotic expression vector containing genomic DNA which codes for the constant regions of human immunoglobulins, transforming eukaryotic 15 cells with said vector(s) and expressing and isolating the antibody.

20 The anti-EGFR scFvs, and above all, the whole anti-EGFR antibodies can be used in diagnosis and therapy of human tumors. Thus, the invention relates to a pharmaceutical composition comprising an anti-EGFR single chain Fv or a whole anti-EGFR antibody as defined above or as defined in the claims.

25

The results and advantages of the present invention can be summarized as follows:

30 Novel mouse anti-EGFR antibodies were isolated from phage-antibody libraries. The new antibodies represented at least four different V_H subgroups and four different V_L subgroups (Kabat et al., *Sequences of proteins of immunological interest*. 5th Eds., U.S. Dept. of Health and Human Services, Bethesda 1991). They showed different pairings and 35 sequences from those used by a mouse MAb isolated using hybridoma technology. Mouse 425 MAb has a $\text{V}_\text{H}2\text{b}$ and $\text{V}_\text{L}4$ pairing that was not

observed in the phage antibodies. The VH of scFv L3 11D had the highest percent identity to 425 VH (84.9%). The majority of the differences were in the CDRs. The $\text{V}\kappa$ of scFv S4 2D had the highest percent identity to 425 $\text{V}\kappa$ (83.2%). Again the majority of differences were in the CDRs, particularly CDR3. In this invention, a variety of new anti-EGFR antibodies were isolated from the phage-antibody libraries and these antibodies all differ from 425 MAb with at least two of the scFvs recognizing a different epitope on EGFR from that recognized by 425 MAb. This is in contrast to a previous report where the antibodies isolated from combinatorial libraries were reported to be very similar to those isolated by hybridoma technology (Caton and Koprowski, *Proc. Natl. Acad. Sci. USA* 1990. 87: 6450).

Of the three phage-antibody libraries, the best library in terms of the number of selection steps required to get high affinity antibodies and in terms of the diversity of high affinity antibodies isolated was the library generated from the draining lymph node. Lymph nodes were selected as a source of RNA for the construction of phage-antibody libraries for two reasons. First, previous work had demonstrated that a higher proportion of B cells producing high affinity IgG antibodies was obtained from the popliteal lymph nodes following immunization via the footpad than from spleens following immunization via the peritoneum (Venn and Dresser, *J. Immunol. Methods* 1987. 102: 95). Second, draining lymph nodes are considered to be a good source for the isolation of human anti-tumor antibodies. Thus, the isolation of mouse anti-EGFR antibodies from the popliteal lymph node of a footpad-immunized mouse was a model for the isolation of human anti-EGFR antibodies from the axillary lymph nodes of a breast cancer patient. The feasibility of preparing good size libraries from small amounts of

lymph node material and of then isolating high affinity antibodies from the libraries was demonstrated.

Although mouse anti-EGFR antibodies were isolated from all three phage-antibody libraries, it is not clear that any of the newly isolated antibodies have higher affinities than the mouse 425 MAb isolated using hybridoma technology. In the first analyses, the phage-antibody derived scFvs appeared to bind to EGFR better than the scFv constructed from 425 MAb (Fig. 2). In other experiments with chimeric whole antibody molecules, one of the chimeric antibodies (S4 2D) showed an affinity for EGFR that equalled that of the chimeric 425 antibody. The second chimeric antibody (L3 11D) had an affinity that was four-fold lower than that of chimeric 425 antibody (Fig. 4). Binding data obtained using scFvs was misleading probably because preparations of scFvs can contain mixtures of monomers and dimers (Griffiths et al., *EMBO J.* 1993, 12: 725). In contrast, chimeric IgG antibodies are not expected to form dimers and the chimeric L3 11D and S4 2D antibodies were demonstrated to be the size expected for bivalent, monomeric chimeric IgG antibodies. Analysis of affinity-purified preparations of 425, L3 11D, and S4 2D scFvs, however, showed that these preparations of scFvs did contain monomeric, dimeric, and other multimeric forms. In addition, the relative proportions of monomeric and multimeric forms varied for each scFv. The 425 scFv had the lowest percentage of dimeric forms. As predicted, the dimeric and particularly the larger multimeric forms showed stronger binding to purified EGFR than the monomeric form. It appears that 425 scFv has a weaker tendency to dimerize than some of the newly isolated scFvs.

35 Although the expression of antibody fragments on the surface of phage particles forms the basis of a powerful method for rapidly selecting for

antibodies with the desired specificities, neither phage antibodies nor the antibody fragments themselves (scFvs or Fabs) are likely to be the desired end product. Further it is demonstrated how the mouse scFvs isolated from phage libraries can be readily converted into whole antibody molecules. In this case, the mouse variable regions were joined to human constant regions to create partially humanized chimeric antibodies.

These results show that it is possible to use phage-antibody technology to isolate a variety of anti-EGFR antibody fragments from immunized mice. Whole antibody molecules with the desired constant regions can then be constructed from the antibody fragments. In some cases, hybridoma technology may still be the method of choice for isolating monoclonal antibodies from mice. If a highly immunogenic antigen is available and if a few hybridoma cell lines producing one or a few different anti-antigen antibodies are adequate, then there is probably little reason to consider phage-antibody technology. If, however, special immunization protocols such as footpad injections would be advantageous in generating high affinity antibodies, or if a large number of antibodies against a variety of epitopes on the antigen are required, or if antibodies directed against a very discreet, and possibly less immunogenic, epitope are required, then phage-antibody technology may be the method of choice. Also, if further genetic engineering of the antibodies is anticipated, then the phage-antibody technology is advantageous in that the antibody genes have already been cloned.

The present approach of combining *in vitro* immunization with a particulate antigen and PCR-cloning technology has generated scFv fragments which reacted with EGFR and did not cross-react with other antigens. The immunization protocol reported here depends on the

antigen presentation, which is not soluble but is a membrane vesicle preparation, and on the culture medium itself, which is devoid of FCS. Both methodologies have been reported as a means of increasing the efficiency of *in vitro* immunization by making the antigen available to the antigen-presenting cells (e.g. Brams, P. et al.; J. Immunol. Methods, 1987. 98: 11).

The results obtained with MTC are in agree with previous papers (e. g. 10 Borrebaeck, C.A.K. and Möller, S.A; J. Immunol., 1986. 136: 3710; Möller, S.A. and Borrebaeck, C.A.K., in Borrebaeck, C.A.K. (Eds.), *In Vitro* 15 Immunization in Hybridoma Technology, Elsevier Science Publishers B.V., Amsterdam 1988, p. 3.) which propose the use of MTC supernatants as a source of lymphokines for improving the *in vitro* immunization process. 15 The membrane vesicle preparation should be envisaged as a poly- 20 antigen since many different antigenic determinants are present in such vesicles. For this reason, it would appear that they induce a certain level of polyclonal activation. We have ruled this out because the anti-EGFR specific response was clearly different from the response obtained after a standard polyclonal activator.

Instead of immortalizing the B-cells after *in vitro* immunizations, we have 25 used the molecular strategy of immortalizing the antibody V_H and V_L genes. These monoclonal antibody fragments were expressed and produced in bacteria. The phage display system is a powerful method to 30 isolate antibody fragments against specific antigens. The presence of a stop codon between the antibody fragment and the g3p coat protein permits the switch between surface display and secretion as a soluble scFv fragment using suppressor or non-suppressor strains (Hoogenboom et al., Nucl. Acids Res. 1991. 19: 4133).

35 Due to the increase of specific response and mRNA levels in *in vitro* antigen stimulated B-cells, *in vitro* immunization contributes to the

isolation of antibody fragments with high specificities to the antigen. After two rounds of selection, 100% of the clones were positive for binding EGFR. In contrast, clones derived from *in vivo* immunization processes were 100 % positive only after four rounds of selection (Kettleborough, et al., EP 94104160 and Eur. J. Immunol. 1994. 24: 952).

The use of phage display libraries from naive antibody genes might allow specific human antibody fragments to be made without immunization or 10 after *in vitro* immunization. Antibody fragments can be directly produced in bacteria, thus in a simple, fast and economic way.

BIOLOGICAL MATERIALS AND GENERAL METHODS

15 Microorganisms, cell lines, plasmids, phagemids, promoters, resistance markers, replication origins or other fragments of vectors which are mentioned in this application are commercially or otherwise generally available. Provided that no other information in the 20 application is given, they are used only as examples and are not essential according to the invention and can be replaced by other suitable tools and biological materials, respectively.

25 Bacterial hosts are preferably used for cloning the scFvs and for producing the scFv proteins. Examples for these hosts are: E. coli or Bacillus.

30 Eukaryotic hosts like COS, CHO or yeasts, for example, are preferred in order to produce the whole anti-EGFR-antibodies according to the invention.

35

The techniques which are essential according to the invention are described in detail in the specification. Other techniques which are not described in detail correspond to known standard methods which are well known to a person skilled in the art, or are described more in detail in the cited references and patent applications and in the standard literature.

10 **Brief Description of the Figures:**

15 Figure 1. Amino acid sequences of scFvs isolated from phage-antibody libraries. (A) scFvs from the lymph node library. (B) scFvs from the spleen library. Complementarity determining regions (CDRs) and framework regions (FRs) are indicated.

20 Figure 2. Binding of scFvs to EGFR. The concentrations of scFvs in bacterial supernatants were estimated and the scFvs tested by ELISA for binding to purified EGFR. (A) scFvs from the lymph node library. (B) scFvs from the spleen library. P1 (positive control) is the scFv derived from MAb 425. L1 and S1 (negative controls) are non-binding scFvs from the pre-selected lymph node and spleen libraries.

25 Figure 3. Intermediate vectors used to reconstruct the variable regions for expression in mammalian cells. (A) V_H vector. (B) V_K vector.

30 Figure 4. Binding of chimeric whole antibodies to EGFR. The concentrations of antibodies in COS cell supernatants were determined by ELISA and the antibodies tested by ELISA for binding to purified EGFR.

Figure 5. DNA and amino acid sequence of scFv No. L2 11C.

(A): Light chain; (B): Heavy chain.

Amino acid positions:

- 5 (A) FR-1: 1 - 23, CDR-1: 24 - 34,
 FR-2: 35 - 49, CDR-2: 50 - 56,
 FR-3: 57 - 88, CDR-3: 89 - 97,
 FR-4: 98 - 109.

- 10 (B) FR-1: 1 - 30, CDR-1: 31 - 35,
 FR-2: 36 - 49, CDR-2: 50 - 66,
 FR-3: 67 - 98, CDR-3: 99 - 108,
15 FR-4: 109 - 119.

Figure 6. DNA and amino acid sequence of scFv No. L2 12B.

(A): Light chain; (B): Heavy chain.

Amino acid positions:

- 20 (A) FR-1: 1 - 23, CDR-1: 24 - 38,
 FR-2: 39 - 49, CDR-2: 50 - 56,
 FR-3: 57 - 88, CDR-3: 89 - 97,
 FR-4: 98 - 109.

- 25 (B) FR-1: 1 - 30, CDR-1: 31 - 35,
 FR-2: 36 - 49, CDR-2: 50 - 66,
 FR-3: 67 - 98, CDR-3: 99 - 108,
 FR-4: 109 - 119.

30 Figure 7. DNA and amino acid sequence of scFv No. L3 11D.

(A): Light chain; (B): Heavy chain.

The amino acid positions of the FRs and CDRs correspond to those given in Fig. 6.

Figure 8. DNA and amino acid sequence of scFv No. S4 2D

(A): Light chain; (B): Heavy chain.

Amino acid positions:

5 (A) FR-1: 1 - 23, CDR-1: 24 - 35,
 FR-2: 36 - 50, CDR-2: 51 - 57,
 FR-3: 58 - 89, CDR-3: 90 - 98,
 FR-4: 99 - 110

10 (B) FR-1: 1 - 30, CDR-1: 31 - 35,
 FR-2: 36 - 49, CDR-2: 50 - 66,
 FR-3: 67 - 98, CDR-3: 99 - 107,
15 FR-4: 108 - 118.

20 The sequences of Figures 5 - 8 are also given in the attached
 Sequence Listing which is part of the disclosure of this invention.

25

DETAILED DESCRIPTION OF THE INVENTION

25

(1) Construction and screening of phage-antibody libraries

30

Three phage-antibody libraries were constructed, one from the spleen
of a mouse immunized with human carcinoma cell line A431 (8.8×10^5
members), one from the popliteal lymph node of a mouse immunized
in the footpad with purified EGFR (6.5×10^6 members), and one from
mouse lymphocytes immunized *in vitro* with A431 vesicles (1.1×10^5
members), (details of construction of A431 vesicles and *in vitro*-
immunization are given in Examples 1, 2). Prior to selection, at least
35 46 clones from each library were analyzed by BstNI fingerprinting

(Clackson et al, *Nature* 1991, 352: 624) to determine the diversity of the repertoires. A wide range of digestion patterns was observed. Also prior to selection, scFvs from 96 clones from each library were tested by ELISA for binding to EGFR. None of the scFvs from the spleen and lymph node library bound to EGFR. One of the scFvs from the *in vitro* immunized library bound to EGFR. After one round of selection using EGFR-coated immunotubes, a clear enrichment for EGFR-binding scFvs was observed with the lymph node library and with the *in vitro* immunized library. A second round of selection was needed before any EGFR-binding scFvs were detected from the spleen library. By the third round of selection, the majority of the scFvs from the lymph node and *in vitro* immunized libraries were positive for binding to EGFR. After a fourth round of selection with the spleen library, the majority of the scFvs were positive for binding to EGFR (Table 1).

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Table 1. Percent of EGFR-binding clones after each round of selection.

	Lymph Node Library	Splee n Libra ry	<i>In vitro</i> Immuniz ed Cells Library
Pre-selection	0	0	1
First round	77	0	84
Second round	86	26	100
Third round	90	77	100
Fourth round	not tested	97	not tested

(2) Sequence analysis of EGFR-binding clones

After each round of selection, scFv inserts from EGFR-binding clones were analyzed by BstNI fingerprinting (Clackson et al, *Nature* 1991, 352: 624). It became clear that there was an enrichment for certain digestion patterns. Clones with different BstNI fingerprints were chosen from the second and third rounds of selection of the lymph node library and from the third and fourth rounds of the spleen library for DNA sequencing of the V_Hs and V_Ks. Clones from later rounds of selection

were analyzed because higher affinity antibodies were expected to be in the later rounds (Clackson et al, *Nature* 1991. 352: 624).

Sixteen clones from the lymph node library were sequenced and six different scFvs were obtained (Figure 1). Five of these were pairings of unique V_H s and V_K s. The sixth was a variation of a previously occurring V_H with six amino acid changes, five of which were in framework region (FR) 1. Two of these changes can be attributed to the use of the degenerate VH1BACKSF1 primer (Hoogenboom et al., *Nucl. Acids Res.* 1991. 19: 4133). The others may be a result of PCR errors. The V_H s were classified into two subgroups, V_H 2b and V_H 3d, while the V_K s fell into four subgroups, V_K 3, V_K 4, V_K 5, and V_K 6 (Kabat et al., *Sequences of proteins of immunological interest*. 5th Eds., U.S. Dept. of Health and Human Services, Bethesda 1991). Ten individual clones from the spleen library were sequenced and four different scFvs were found. Three of these were pairings of unique V_H s and V_K s while the fourth was similar to one of the previous pairings with only two amino acid differences in V_H , one of which occurred in complementarity determining region (CDR) 2, and two amino acid differences in V_K . Classification into subgroups revealed V_H s from subgroups V_H 2a, V_H 2c, and V_H 3d and V_K s from subgroups V_K 3 and V_K 4. Comparison of the scFvs obtained from the lymph node and spleen libraries revealed only one scFv that was common to both libraries, scFv L3 10A/scFv S4 10H (Figure 1). This clone appeared to bind strongly to EGFR when tested by ELISA. While much care was taken to eliminate any cross-contamination between libraries, it is difficult to rule out minor contamination with a strongly-binding EGFR clone. However, considering the inbred nature of Balb/c mice, it is possible that the same scFv arose independently from two different libraries.

(3) Analysis of the affinity and specificity of binding to EGFR

Based on good binding to antigen and diversity in DNA sequences, several scFvs derived from the lymph node and spleen libraries were chosen for further analysis. These scFvs were analyzed by ELISA for binding to purified EGFR, binding to irrelevant antigens, and binding to tumor cell lines that did or did not express EGFR. As a positive control, scFvs were prepared from mouse 425 MAb (P1). As negative controls, scFvs were prepared from phage antibodies isolated from the lymph node and spleen libraries prior to selection (L1 and S1, respectively). The concentration of scFvs was determined by comparing dilutions of the scFvs to be tested with dilutions of a purified scFv of known concentration in a Western blot.

The scFvs were tested by ELISA for binding to purified EGFR and the results plotted (Figure 2). It was possible to rank the scFvs with respect to their binding to EGFR. These rankings were reproducible between experiments. The scFvs that bound most strongly to EGFR were L2 1C and L3 10A from the lymph node library and S4 10H from the spleen library. As described previously, scFvs L3 10A and S4 10H have the same DNA sequences. A scFv (S4 5A) that was very similar to scFv S4 10H, with two amino acid changes in V_H and two in V_K, consistently gave a lower ranking than S4 10H. In contrast, the differences in sequence observed between L2 12B and L3 11D did not appear to have a pronounced effect on the binding. Of the scFvs isolated only two, L2 8C and L2 11C, appeared to bind less well than scFv 425.

The scFvs were tested by ELISA for binding to plastic and to a panel of unrelated proteins (ovalbumin, hen egg lysozyme, cytochrome c, glyceraldehyde 3-phosphate dehydrogenase, CBA albumin, and BSA). None of the scFvs gave a signal above background.

The scFvs were tested by ELISA for binding to three tumor cell lines. Cell lines A431 and MDA MB 468 are EGFR-bearing tumor cells isolated from the vulva and breast, respectively. Cell line SK-MEL-23 is a ganglioside-bearing melanoma cell line and was included as a negative control. Of the ten scFvs tested, only four bound to both purified EGFR and EGFR-bearing tumor cells (L2 12B, L3 11D, L2 11C, and S4 2D, Figures 5 - 8). No binding to SK-MEL-23 cells was detected. There are several possible explanations for this surprising result. One may be that the EGFR that was used for immunization, selection, and ELISA was secreted EGFR-related protein (Weber et al., *Science* 1984, 224: 294). This protein has an additional 17 amino acids at the C-terminus (Günther et al., *J. Biol. Chem.* 1990, 265: 22082). The scFvs were tested by ELISA for binding to this 17 amino acid peptide and no binding was observed. It is possible that the secreted EGFR-related protein and the EGFR on the tumor cell surface have differences in conformation or glycosylation.

To further investigate binding to tumor cells, three scFvs (L2 11A, L3 11D and S4 2D) were purified and analyzed for binding to A431 tumor cells by flow cytometry. The 425 scFv was used as a positive control. Of the three scFvs tested, only L3 11D and S4 2D bound to A431 cells. These two scFvs had similar binding profiles to scFv 425.

Purified scFvs prepared from two of the isolates that bound to both EGFR and EGFR-bearing tumor cells (L3 11D and S4 2D) were tested in competition binding assays with mouse 425 MAb. While purified scFv 425 was able to inhibit mouse 425 MAb from binding to EGFR over a given concentration range, scFvs L3 11D and S4 2D did not inhibit mouse 425 MAb from binding to EGFR at these concentrations.

These two scFvs appear to recognize an epitope on EGFR that is different from that recognized by mouse 425 MAb.

(4) Chimeric whole antibodies derived from scFvs.

Two scFvs (L3 11D and S4 2D) were selected for conversion into whole antibody molecules. DNAs coding for the mouse V_H s and V_L s were cloned into intermediate vectors containing DNA sequences coding for immunoglobulin leader sequences and splice donor signals (Fig. 3). The positioning of the cloning sites in the V_H intermediate vector meant that the first residue of the V_H was changed from aspartic acid to glutamic acid. From the intermediate vectors, DNA fragments containing the V_H s and V_L s, now joined to leader and splice donor sequences, were cloned into mammalian cell expression vectors containing DNAs coding for either human gamma-1 constant region or human kappa constant region (Maeda et al., *Hum. Antibod. Hybridsomas* 1991. 2: 124). For each chimeric antibody, the heavy chain and light chain expression vectors were co-transfected into COS cells. As a positive control, cells were also co-transfected with heavy and light chain expression vectors coding for chimeric 425 antibody (Kettleborough et al., *Protein Eng.* 1991. 4: 773). Medium was collected from the cells and analyzed by ELISA to determine the concentration of antibody present and the ability of the antibody to bind to EGFR (Fig. 4). When the antibody concentration required to achieve half-maximum binding to antigen were compared, chimeric S4 2D antibody bound to EGFR equally as well as chimeric 425 antibody. Chimeric L3 11D antibody, however, bound to EGFR approximately four-fold less well than chimeric 425 antibody. The affinity of chimeric 425 antibody (Kettleborough et al., *Protein Eng.* 1991. 4: 773) has been determined by competition binding analysis to be $1.9 \times 10^8 \text{ M}^{-1}$. These results were surprising because previous data analyzing the scFvs had indicated that scFvs S4 2D and L3 11D both bound to EGFR better

than scFv 425 (Fig. 2). Protein A-purified samples of chimeric L3 11D and S4 2D antibodies were analyzed by SDS-PAGE under reducing and non-reducing conditions. Chimeric L3 11D and S4 2D antibodies were also tested by flow cytometry for binding to A431 and SK-MEL-23 cells. Both chimeric antibodies bound well to the EGFR-expressing A431 cells and did not bind to the EGFR-negative SK-MEL-23 cells.

10 **(5) Therapeutic and diagnostic use**

The antibody fragments and whole antibodies according to the invention can be administered to human patients for therapy. Therefore, it is an object of the invention to provide a pharmaceutical formulation comprising as active ingredient at least one antibody or antibody fragment as defined above and in the claims, associated with one or more pharmaceutically acceptable carrier, excipient or diluent therefore.

20 Typically the antibody of this invention will be injected intravenously or parenterally. Generally, the dosage ranges for the administration of the antibodies fragments are large enough to produce the desired tumor suppressing and tumor lysing effect. The dosage will depend on age, condition, sex and extent of the disease in the patient and can vary from 0.1 mg/kg to 200 mg/kg, preferably from 0.1 mg/kg to 100 mg/kg/dose in one or more doses administered daily, for one or several days.

25 Preparations for parenteral administration includes sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oils, and injectable organic esters such as ethyl oleate and other solvents known in the art which are suitable for these

purposes. The antibodies of this invention can be used in a composition comprising a physiologically acceptable carrier. Examples of such suitable carriers are saline, PBS, Ringer's solution, or lactated Ringer's solution. Preservatives and other additives such as antibiotics, antioxidants, and chelating agents may also be present in the pharmaceutical formulations.

5 The antibody (fragment) can also be conjugated according to known methods to cytokines such as IL-2 in order to support their cytotoxicity.

10 The pharmaceutical formulations of the present invention are suitable for the treatment of all kinds of tumors, including melanomas, gliomas and carcinomas, as well as tumors of the circulating system and solid tumors.

15 For diagnostic purposes the antibody can be conjugated, for example, to a radio-opaque dye or can be radiolabelled. A preferred labelling method is the Iodogen method. Preferably the antibody will be administered as F(ab')₂ or scFv fragments for diagnostic purposes. This provides superior results so that background subtraction is unnecessary.

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EXAMPLE 1: A431 vesicles

Shed membrane vesicle preparations were obtained as previously described by (Cohen et al., *J. Biol. Chem.* 1982. 257: 1523; Yeaton et al., *J. Biol. Chem.* 1983. 258: 9254) with some modifications. Confluent flasks containing A431 cells were washed with PBS containing calcium

and magnesium. Hypotonic PBS was added and flasks were shaken for 15 minutes. Cells were then washed with vesiculation buffer (100 mM NaCl, 50 mM Na₂HPO₄, 5 mM KCl, 0.5 mM MgSO₂, pH 8.5). Vesiculation buffer was added and flasks were kept in agitation at room temperature and at 37°C. Then, buffer was decanted through metallic screen into 50 ml tubes in ice and centrifuged for 5 minutes at 150 x g at 4°C. The pellet was discarded and the supernatant was ultracentrifuged at 39,000 rpm for 90 minutes. The final pellets were resuspended in 10 mM Hepes buffer (pH 7.4). To analyze EGFR from vesicles, samples were precipitated with 9 volumes of ethanol resuspended with 0.08 M Tris, pH 6.8, and then SDS-PAGE was carried out with MAbs 425 as standard.

The protein content of the preparations was quantitated by a modified Coomassie Plus method using BSA as a standard and read at 595 nm. To analyze EGFR from vesicles, samples were precipitated with 9 volumes of ethanol (overnight at 4°C). The pellet was resuspended with Tris (0.08 M, pH 6.8) and then a SDS-PAGE was run (5% stacking gel; 1h, 35 mA; 10% running gel; 2.5 h; 40 mA). Samples and standard were in duplicate. One of them was stained with Coomassie Blue and the other was blotted onto nitrocellulose sheets (12 V; 16 h at 4°C) and treated with mouse mAb 425 (anti-EGFR) and anti-mouse IgG antibody conjugated to alkaline phosphatase.

Three media were used in the *in vitro* immunizations. Medium-1 (M1), Medium-2 (M2) and Mixed Thymocyte Culture medium (MTC). M1 consisted of HL1 (Ventrex Laboratories, USA) supplemented with 50 mM 2-mercaptoethanol and 2 mM L-Glutamine (Gibco). M2 consisted of HL1 supplemented with 50 mM 2-mercaptoethanol; 40 U/ml IL-2 (Genzyme); 20 mg/ml Adjuvant Peptide (Sigma); 2 mM L-glutamine; 100 U/ml penicillin (Gibco); 100 mg/ml streptomycin (Gibco). 4% or 20% of FCS

(Biological Industries) was added to M2. MTC was prepared as described by Vaux (1). Briefly single cell suspensions of thymuses of three-week-old Balb/c and C57/BL-1 mice were prepared by pressing the thymus glands through a sterile 50-mesh screen. The cell sus-pension was collected, washed twice in HBSS and the number of viable cells was determined by trypan blue exclusion. Thymocytes were then cultured at a density of 2.5×10^6 thymocytes of each strain per ml in HL1 medium containing 4% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. After 48 hours, the supernatant was recovered, filtered through a 0.22 mm filter, and stored at -70°C.

A suspension of splenocytes from non-immunized eight-week old BALB/c mice was obtained as described for thymocytes. Viability was determined by trypan blue exclusion.

EXAMPLE 2: In vitro immunization and screening

Three media were used in the *in vitro* immunizations. Medium-1 (M1), Medium-2 (M2) and Mixed Thymocyte Culture medium (MTC). M1 consisted of HL1 (Ventrex Laboratories, USA) supplemented with 50 mM 2-mercaptoethanol and 2 mM L-Glutamine (Gibco). M2 consisted of HL1 supplemented with 50 mM 2-mercaptoethanol; 40 U/ml IL-2 (Genzyme); 20 mg/ml Adjuvant Peptide (Sigma); 2 mM L-glutamine; 100 U/ml penicillin (Gibco); 100 mg/ml streptomycin (Gibco). 4% or 20% of FCS (Biological Industries) was added to M2. MTC was prepared as described by Vaux (1). Briefly single cell suspensions of thymuses of three-week-old Balb/c and C57/BL-1 mice were prepared by pressing the thymus glands through a sterile 50-mesh screen. The cell sus-pension was collected, washed twice in HBSS and the number of viable cells was determined by trypan blue exclusion. Thymocytes were then cultured at a density of 2.5×10^6 thymocytes of each strain per ml in HL1 medium

containing 4% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 mg/ml streptomycin. After 48 hours, the supernatant was recovered, filtered through a 0.22 mm filter, and stored at -70°C.

5 A suspension of splenocytes from non-immunized eight-week old BALB/c mice was obtained as described for thymocytes. Viability was determined by trypan blue exclusion.

Single cell suspensions from thymuses of three-week-old Balb/c and
10 C57/BL-1 mice were obtained by pressing the thymus glands through a sterile 50-mesh screen. The cell suspension was collected, washed with HBSS and the number of viable cells was determined by trypan blue exclusion. Thymocytes were then cultured at a density of 2.5×10^6
15 thymocytes of each strain per ml in HL1-medium containing 4% FCS, 2 mM L-glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin. After 48 hours the supernatant was recovered, filtered and stored. A suspension of splenocytes from non-immunized eight-week-old
20 BALB/c mice was obtained as described for thymocytes. Viability was determined by trypan blue exclusion.

In vitro immunizations were performed in 6-well plates (Costar). Wells containing 10^7 splenocytes in 3.5 ml of M1-medium (consisting of
25 HL1-medium, Ventrex Laboratories, USA, supplemented with 50 µM 2-mercaptoethanol and 2 mM L-glutamine (Gibco)) were incubated (37°C, 5% CO₂) with vesicles bearing EGFR at the desired concentration. Vesicles from cells not expressing EGFR or PBS were
30 added in control wells. After some hours , 3.5ml of M2-medium (consisting of HL1 supplemented with 50 µM 2-mercaptoethanol, 40 U/ml IL-2 (Genzyme), 20 µg/ml adjuvant peptide (SIGMA), 2 mM L-glutamine, 100 U/ml penicillin (Gibco), 100 µg/ml streptomycin
35 (Gibco)) containing 4% or 10% FCS (Biological Industries) was added to each well. In some experiments M2 was replaced by MTC-medium

(mixed thymocyte culture medium (Vaux et al., *Nature* 1988, 336: 36) supplemented with adjuvant peptide (20 μ g/ml) and IL-2 (40 U/ml)

5 (Note that the final concentration of FCS, IL2 and adjuvant peptide in culture is 50% reduced). Cells were incubated for 72, 96, 120 or 144 h in the same conditions and, finally, the cells were tested for the presence of specific immunoglobulin or processed for RNA isolation.

Screening was carried out with purified antigens or A431 fixed cells.

10 The procedure was essentially as previously described (Carroll et al., *Hybridoma* 1990, 9: 81) with some modifications. Briefly, sterile 96-well plates (Nunc, Maxisorb) were coated overnight with purified EGFR (2.5 μ g/ml), GD₃ ganglioside (2 μ g/ml), or RNase (10 μ g/ml) in PBS.

15 When A431 cells were used as antigen, cells were cultured in 96-well plates until confluent and fixed with 0.1 % glutaraldehyde. *In vitro* immunized lymphocytes were washed and resuspended in HL1 medium supplemented with 2 % FCS and 2 mM of L-glutamine at

20 5x10⁵ cells/ml and 1x10⁵ cells were added to each well and incubated (37°C, 5% CO₂) for 48 h. Sixteen duplicates of each group were done. Lymphocytes were then removed by washing 5 times in PBS containing 0.1 % Tween-20. Specific immunoglobulins were

25 detected using peroxidase labelled rabbit anti-mouse immunoglobulin (Dako) (1 hour, 37°C). 2,2'-Azino-bis(3-ethylbenz-thiazoline-6-sulfonicacid)-diammonium salt (ABTS) (Sigma) in citrate-phosphate buffer (0.55 mg/ml) was used as substrate.

30 **EXAMPLE 3: Library construction**

Three libraries were constructed from RNA prepared from the spleen of a mouse immunized intraperitoneally with A431 cells (Murthy et al., *Arch. Biochem. Biophys.* 1987, 252: 549) from the popliteal lymph node of a mouse immunized in the footpad with purified EGFR, and from mouse cells immunized *in vitro* with A431 vesicles. First-strand

cDNA was synthesized. The V_H and V_K genes were PCR-amplified and assembled (Clackson et al., *Nature* 1991. 352: 624). Using PCR, NotI and SfiI restriction sites were appended and the scFvs cloned into the phagemid vector pHEN1 (Hoogenboom et al., *Nucl. Acids Res.* 1991. 19: 4133). The ligation mixtures were electroporated into *E. coli* cells and the resulting colonies scraped into medium to generate library stocks (Marks et al., *J. Mol. Biol.* 1991. 222: 581).

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EXAMPLE 4: Library screening

Phage antibodies were rescued from the libraries using M13K07 helper phage (Promega, Madison, WI) (Marks et al., *J. Mol. Biol.* 1991. 222: 581). Immunotubes (Nunc, Life Sciences, Paisley, UK) were coated with 4 ml of 2.5 μ g/ml EGFR in PBS overnight. After three washes with PBS, tubes were incubated at 37°C for at least 1 h in PBS containing 2% milk powder (PBSM). The phage (10^{12} to 10^{13}) were resuspended in 4 ml PBSM and incubated in the EGFR-coated tube for 1 h at room temperature. The tube was washed 20 times with PBS, 0.1% Tween and 20 times with PBS. Bound phage were eluted after a 10 min incubation in 1 ml of 0.1 M triethylamine with end-over-end mixing. The eluted phage were neutralized by the addition of 0.5 ml of 1 M Tris-HCl, pH 7.5 and used to infect log-phase *E. coli* TG1 cells. Infected cells were plated and individual colonies picked for small-scale induction of scFvs. The remaining colonies were scraped into medium and an aliquot used to prepare phage for the next round of screening.

35 EXAMPLE 5: Production and analysis of scFvs

Soluble scFvs were produced in *E. coli* HB2151 as previously described (e.g. Kettleborough et al., *i. c.*). The scFv concentrations in the bacterial supernatants were estimated using a purified scFv preparation of known concentration as a standard. Supernatants were filtered and sodium azide added to 0.1%. Serial dilutions of the supernatants and of the standard were spotted onto Immobilon-PVDF filters (Millipore, Watford, UK) using a 96-well manifold. The filters were treated as for a Western blot (Towbin et al., *Proc. Natl. Acad. Sci. USA* 1979, 76: 4350).

10 The scFvs were detected using an antibody (9E10) directed against the C-terminal tag (Munro and Pelham, *Cell* 1986, 46: 291) followed by a peroxidase-conjugated goat anti-mouse IgG and IgM antibody (Jackson ImmunoResearch Lab Inc., West Grove, PA). The reactions were developed using the ECL system (Amersham, Aylesbury, UK). Pre-flashed autoradiographs were scanned using a densitometer. A standard curve was prepared and used to estimate the scFv concentrations in the supernatants.

15 Antigen-binding ELISAs were carried out with EGFR-coated plates (2.5 µg/ml). Supernatants containing scFvs were diluted in PBSM and added to the plates. Bound scFvs were detected using 9E10 antibody as described above. Supernatants were also tested for binding to a panel of unrelated proteins and plastic. ELISA plates were coated overnight at 100 µg/ml with ovalbumin, hen egg lysozyme, cytochrome c, glyceraldehyde 3-phosphate dehydrogenase, murine albumin (CBA strain), and BSA. Undiluted supernatants containing 2% milk powder were added in duplicate to the coated plates and bound scFvs detected as described above.

20 Cell-binding ELISAs were carried out using tumor cell lines, A431 (ATCC CRL 1555), MDA MB 468 (ATCC HTB 132), and SK-MEL-23 (negative control). Cells were grown to confluence in poly-D-lysine-

treated 96 well tissue culture trays (Nunc). Cells were washed with DMEM and blocked at 37°C for 2 h with PBS containing 2.5% BSA. After aspiration, supernatants were added to each well together with an equal volume of 2xYT media containing 4% milk powder and incubated at 4°C for 1 h. Bound scFvs were detected as described above.

A competition-based ELISA was carried out by pre-incubating EGFR-coated ELISA plates with 50 µl of purified scFv (100 µg/ml) for 10 min. Mouse MAb 425 (50 µl) was then added to give concentrations of 3.13 to 200 ng/ml. Following incubation and washing, bound mouse MAb 425 was detected using peroxidase-conjugated goat anti-mouse IgG and IgM antibody.

EXAMPLE 6: DNA analysis

For BstNI fingerprinting, the scFv inserts from individual clones were amplified by PCR and the products digested with BstNI (Clackson et al., *Nature* 1991, 352: 624). DNA was sequenced using a Sequenase kit (United States Biochemical, Cleveland, OH).

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EXAMPLE 7: Purification of scFvs

Bacterial supernatants were clarified by centrifugation and filtration through 0.2µm filters before loading onto a 1 ml column of purified EGFR (5 mg) coupled to cyanogen bromide-activated Sepharose 4B (Pharmacia, Uppsala, Sweden). The column was washed with 30 ml of PBS followed by 5 ml 0.2M glycine, pH 5.0. The scFvs were eluted

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with 0.2M glycine/HCl, pH 2.8. The eluate was neutralized with 10x PBS. Protein-containing fractions were pooled and the buffer changed by ultrafiltration (Amicon, Stonehouse, UK) to PBS containing 1% BSA and 0.05% sodium azide.

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EXAMPLE 8: FACS analysis of purified scFvs

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A431 cells were trypsinized and incubated in DMEM containing 10% FCS. Cells were washed twice with cold DMEM and filtered through a 45 µm screen. Cells (10^6) were incubated on ice for 30 min in 50 µl PBS, 1% BSA, with purified scFvs. After two washes with cold PBS, bound scFvs were detected using 50 µl FITC-conjugated 9E10 antibody (100 µg/ml). After 30 min on ice, cells were washed once with PBS, fixed in PBS containing 1% formaldehyde, and analyzed using a FACSCAN (Becton-Dickinson, Cowley, UK).

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EXAMPLE 9: Construction, analysis and expression of whole chimeric antibodies

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Using PstI and BstEII sites, DNAs coding for the V_H s of the selected scFvs were subcloned into an intermediate V_H vector containing a eukaryotic leader sequence derived from human antibody HG3 CL (Rechavi et al., *Proc. Natl. Acad. Sci. USA* 1983, 80: 855) and a splice donor site (Fig. 3). The DNAs coding for the V_k s were adapted for insertion into an intermediate V_k vector using PCR primers to incorporate Xhol and SstI sites at the 5'- and 3'- ends (V_kFor: 5'-CCG TTT CAG CTC GAG CTT GGT CCC-3', V_kBack: 5'-GAC ATT GAG CTC ACC CAG TCT CCA-3').

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The SstI-Xhol fragments were cloned into the intermediate V_k vector containing a eukaryotic leader sequence derived from reshaped

human CAMPATH-1 light chain (Riechmann et al., *Nature* 1988. 332: 21) and a splice donor site (Fig. 3). The DNAs coding for the variable regions plus eukaryotic flanking regions were cloned as HindIII-BamHI fragments into mammalian cell expression vectors containing genomic DNAs coding for human gamma-1 constant region or human kappa constant region (Maeda et al., *Hum. Antibod. Hybridomas* 1991. 2: 124). The heavy and light chain expression vectors were electroporated into COS cells. After 72 h, medium was collected and the chimeric anti-EGFR antibodies analyzed by ELISA (Kettleborough et al., *Protein Eng.* 1991. 4: 773).

15 **EXAMPLE 10: Production of sc Fvs derived from in vitro immunized cells.**

The methods disclosed below are slight modifications of the methods described above. Immunization, library construction and screening are given in Examples 1 - 4. The following steps are described in detail below:

25 After screening the primary library and the clones derived from the three rounds of panning, some single ampicillin-resistant colonies were selected. Phagemid DNA was prepared by alkaline lysis and used to transfect *E.coli* HB2151, a non-suppressor strain, by heat shock. Colonies were inoculated into 2xTY-Amp-Glu and grown overnight at 30°C. A 5 ml aliquot was used to inoculate 50 ml of 2xTY broth containing 100 mg ampicillin/ml and 0.1% glucose and grown with shaking at 30°C for 1 h (until log-phase). Cells were harvested and expression of soluble scFv was induced by the addition of isopropyl β -D-thiogalactopyranoside (IPTG) to a final concentration of 1 mM (De Bellis, D. and Schwartz, I.; 30 Nucleic Acids Res.; 1990. 18: 1311). Cultures were grown overnight at 30°C
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with shaking. Supernatants containing scFv were taken, clarified by centrifugation and filtration through 0.22 mm filters and tested. Bacterial supernatants were tested for binding to EGFR by ELISA, as described (Kettleborough, et al., EP 94104160 and Eur. J. Immunol. 1994, 24: 952).
5 The specificity of selected scFv fragments was checked by ELISA using plates coated with various proteins related and non-related to EGFR, as well as other antigens and plastic. The antigens used were: RNase, BSA,
10 OVA, GD₃ ganglioside, vitronectin receptor (VNR), platelet glycoprotein IIb/IIIa (GPIIb/IIIa), and disialyl-lacto-N-tetraose (DSLNT). Coating was done overnight at the optimum concentration for each antigen. Coated
15 ELISA plates were blocked for 1 h at 37°C with 1.5% skimmed milk in PBS (w/v). After washing, 100 ml of scFv supernatants were added to the microtiter wells and incubated for 2 h at 37°C. Bound scFv were detected using the anti-c-myc antibody 9E10 (spent culture media from Myc 1-
19E10.2 hybrid) and an alkaline phosphatase-conjugated rabbit anti-mouse antibody (Dako).
20 Three EGFR-bearing tumor cell lines, A431, MDA MB 231 human breast adenocarcinoma (ATCC, HTB 26), and HT29 human colon adenocarcinoma (ATCC, HTB 38), and one non-expressing EGFR cell line, WM164, were used to test the ability of scFv to bind to EGFR on
25 cells by mean FACS analysis and immunofluorescence with unfixed cells. For the indirect immunofluorescence analysis, cells were plated into Terasaki plates (2x10⁴ cells/well) and cultured for 24 h. Cells were then
30 incubated with 20 ml of crude bacterial supernatant containing the scFv fragments for 90 min at room temperature. Incubations with primary antibody (anti-c-myc) and secondary antibody were carried out for 60 min at room temperature. The secondary antibody, FITC-conjugated rabbit anti-mouse antibody (Dako) was diluted 1:20.
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For FACS analysis, 5×10^5 cells were washed with PBS with 1% BSA and 0.1% sodium azide (PBS-BSA) and incubated at 4°C for 20 min with 50 ml of crude bacterial supernatant. After two washes with cold PBS-BSA, bound scFv was detected using anti-c-myc antibody and FITC-conjugated goat anti-mouse antibody (Becton-Dickinson) diluted 1:25 in PBS-BSA. Propidium iodide (PI) was added at a final concentration of 5 mg/ml. Flow cytometry analysis were performed in a EPICS Profile II equipped with an air-cooled argon laser. The 488 nm line (15 mV) was used for the excitation. A 530 nm band pass filter was used to collect FITC emission and a 625 nm band pass filter was used to collect PI emission. Living cells were selected by setting a bitmap on forward and side scatter and by exclusion of PI-stained cells.

The diversity of the primary and selected libraries was determined by PCR amplification of cloned fragments (Güssow, D. Clackson, T; Nucleic Acids Res. 1989. 17: 4000) and analysis of the BstNI digestion pattern (8). Some clones were sequenced using a Sequenase kit (USB) by the dideoxy chain termination method (Sanger, F et al.; Proc. Nat.Acad. Sci., U.S.A. 1977. 74: 5463).

Crude bacterial supernatants (10 ml) were subjected to SDS-PAGE using a 12.5% gel. Western blotting was performed essentially as described by Towbin (Towbin et al. J. Proc.Nat.Acad.Sci., U.S.A. 1979. 76: 4350). Proteins were transferred by electroblotting to Immobilon-P (Millipore) or nitrocellulose (Bio-Rad). The blot was blocked with PBS containing 2% skimmed milk (w/v). scFv fragments were detected using anti-c-myc antibody (9E10), peroxidase-conjugated anti-mouse antibody (Jackson), and an enhanced chemiluminiscence system (ECL, Amersham).

The quantitative analysis of the shed membrane vesicles revealed a total protein concentration of 2.5 mg/ml, of which only 10-14% corresponded to EGFR (Sato et al.; J. Natl. Cancer Inst. 1989. 21: 1601; Yeaton, R et al.,

J. Biol. Chem., 1983, 258: 9254), 250 to 350 ng/ml. Electrophoretic analysis using PAGE-SDS followed by Coomassie-blue staining showed that the vesicles contained a rather complex mixture of proteins. No protein degradation was detected. Western blot analysis revealed that under our experimental conditions complete molecules of EGF receptor were present in the membrane vesicle preparation.

In order to determine the requirements for FCS and lymphokines MTC and M2 containing 20% or 4% FCS were compared. Vesicles bearing EGFR and PBS were used as antigen and control respectively. Splenocytes were incubated in six well plates with or without antigen for 3 h in M1 (serum-free). MTC or M2 was then added and, after 72, 96, 120 or 144 h, screening was carried out using A431 fixed cells. In all experiments, the number of viable cells recovered was between 20 and 40% in agreement with published results (Gavilondo-Cowley, J. et al.; In Vitro Immunization in Hybridoma Technology, Elsevier Science Publishers B.V., Amsterdam 1988, p. 131). The maximum specific response was obtained on day four with MTC; whereas, M2 at 4% or 20% FCS (2% or 10% final concentration) delayed the maximum response until day six (Table 2). However MTC and 10% FCS triggered a non-specific response, probably by polyclonal activation, as could be seen when the results were expressed as the ratio of specific / non-specific response. For further assays we decide to use M2 supplemented with 4% FCS and 6 d of culture.

The presence of EGFR in the surface of vesicles strongly enhanced the response to this antigen. In similar protocols as described above, vesicles from expressing and non-expressing EGFR cell lines were compared. Lymphocytes were cultured with vesicles in M1 for 3 h. Afterwards M2 containing 4% FCS was added. After 6 d, lymphocytes from each group were cultured for 48 h in 96 well plates coated with EGFR, A431-fixed cells, RNase or GD3. As expected, the results of these assays showed a

multispecific pattern of response (Table 3). The reactivity against EGFR was clearly increased in terms of optical density when EGFR-expressing vesicles were used as antigen.

- 5 Taken together, these results suggest that, although immature, there was a measurable antigen-dependent response after *in vitro* immunization which generated several pools of immunized lymphocytes against EGFR suitable for PCR-cloning of variable regions.
- 10 A library of 1.1×10^5 clones was obtained after cloning scFv fragments derived from *in vitro* immunization into the pHEN1 phagemid. This library was generated in parallel with two more libraries providing of *in vivo* immunization. The construction of these phages libraries has been
- 15 described previously (Kettleborough, et al., EP 94104160 and Eur. J. Immunol. 1994. 24: 952).

To select the scFv fragments binding to EGFR, phage were panned using EGFR-coated immunotubes. Eluted phage were used to reinfect a *SupE* strain of *E.coli*. In total, three rounds of selection were carried out. In each 20 round, a tube without antigen was tested in parallel to calculate the background. In the first panning, 1.5×10^{10} phage particles were applied to the immunotube and 6.6×10^4 were eluted from the coated immunotube; whereas, only 200 colonies were obtained from the background population. After the third panning, 1×10^{11} phages were applied and 25 5.6×10^{10} were eluted.

To further characterize the scFv fragments, we selected 22 clones from 30 the phage populations, before selection and after each round of selection.

The diversity of the library was analyzed by the BstNI digestion patterns of 35 the cloned fragments. Prior to selection the library appeared to be extremely diverse. Fingerprinting of binding clones derived after the first

round of selection indicated the presence of several groups with the same restriction pattern.

Clones were selected from different rounds of selection based on their
5 digestion patterns. DNA sequencing revealed the presence of different sequences in most of the selected clones. The length and composition of complementarity determining regions (CDRs) of clones 10D2, 5D3, 10E2, 1B3, 4B3 and 5E2 were different. The most variation was observed in the
10 CDR3s of V_H and V_L sequences. Clones 5D3 and 1E3 were derived from the third round of selection. They bound strongly to EGFR as analyzed by ELISA and flow cytometry and had the same sequence.

Soluble scFv fragments were obtained by growth of the non-suppressor
15 E.coli strain HB 2151 in presence of IPTG.

To verify scFv production, bacterial medium from individual clones, was analyzed by gel electrophoresis. Western blot analysis revealed a clear band around 35,000 kD.

20 Clones with binding activities to EGFR were identified by ELISA. To examine the cross-reactivity of selected clones, ELISA assays using different antigens were carried out. The antigens (EGFR, RNase, BSA, KLH, OVA, GD₃ ganglioside, vitronectin receptor, platelet glycoprotein IIbIIIa, and disialyl-lacto-N-tetraose) were coated into ELISA plates at the optimum concentration (Table 4). No binding to non-EGFR antigens was detected. The scFvs were also tested for binding to three EGFR-bearing tumor cell lines (human epidermoid carcinoma A431, human breast
25 adenocarcionma MDA MB 231 and human colon adenocarcinoma HT 29). WM 164 a human melanoma non-expressing EGFR was used as a negative control. Those that bound to tumor cell lines was tested by indirect immunofluorescence using unfixed cells and quantified by FACS analysis. The use of unfixed cells ensures the natural conformation of the
30 membrane receptors. Positive clones showed a clear fluorescence using
35

A431 cells. Fluorescence with the others EGFR-bearing tumor cell lines was weak. No binding to the negative cell line was detected. The results were confirmed by flow cytometry. Seventeen positive clones and three negative clones were analyzed for binding to A431, MDA MB 231 and HT 5 29 cells by flow cytometry. WM 164 was used as the negative cell line. The 425 scFv (P1 clone) was used as a positive control and the cloning vector (HEN) as a negative control. The results are summarized in Table 10 5. Two clones, 4B2 and 5E2, were positive for binding to EGFR, as analyzed by ELISA, but negative for binding to EGFR-expressing tumor cell lines.

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Table 2. Effect of different media on *in vitro* immunization.^{a)}

		Day of screening against A431								
		3rd day		4th day		5th day		6th day		
		Assay	Antigen	O.D. ^{c)}	Ratio ^{d)}	O.D.	Ratio	O.D.	Ratio	
5	1	Vesicles	0.393	2.11	0.801	3.76	0.784	3.90	0.951	10.3
		PBS	0.186		0.213		0.201		0.092	
10	2	Vesicles	0.527	2.50	0.852	1.76	0.863	2.75	1.168	3.94
		PBS	0.210		0.482		0.313		0.296	
15	3	Vesicles	0.763	1.48	1.169	2.01	1.089	2.07	1.115	1.91
		PBS	0.513		0.581		0.525		0.581	

Assay 1: M1 plus M2, 4% FCS (Final FCS: 2%)

Assay 2: M1 plus M2, 20% FCS (Final FCS: 10%)

Assay 3: A-medium plus MTC, 4% FCS (Final FCS: 2%)

a) BALB/c mouse spleen cells (10^7) were incubated in 3.5 ml of M1 with vesicles from A431 cells or PBS for 3 h in wells of 6 well plates. Afterwards 3.5 ml of MTC or M2 containing 4% or 20% FCS were added and the plates incubated.

At 3, 4, 5 or 6 days *in vitro* immunized lymphocytes were removed from culture medium, washed in HBSS to remove vesicles and seeded in 96 well plates coated with fixed A431 cells, and incubated for 48 h (see Methods).

b) Final concentration of FCS in culture medium.

c) O.D. Optical density read at 405nm. It represents the mean of sixteen wells.

d) Ratio of specific response (vesicles as antigen) / unspecific response (PBS as antigen).

Table 3. Multi-specificity of the response after *in vitro* immunization^(a)

		Screening against				
		Antigen group	A431 CELLS	EGFR	GD3	RNase
5	Assay 1	EGFR +	0.512 ^(b)	0.326	0.140	0.249
		EGFR -	0.427	0.070	0.123	0.304
10	Assay 2	EGFR +	1.430	0.730	0.233	0.670
		EGFR -	0.789	0.195	0.118	0.561

a) Lymphocytes were *in vitro* immunized using either EGFR-expressing vesicles (EGFR+) or non-EGFR expressing vesicles (EGFR-). After six days of incubation, cells were removed from culture and screened against the above mentioned antigens.

b) Response is expressed as optical density (405 nm).

Table 4. Cross-reactivity of selected scFv fragments against several antigens^(a)

ANTIGEN ^(b)	COATING [mg/ml]	RESULT
EGFR	2.5	+
RNase	10	-
BSA	10	-
KLH	10	-
OVA	10	-
GD ₃ ganglioside	2	-
VNR	1	-
GPIIbIIIa	1	-
DSLNT	5	-

a) ELISA assays were performed as described.

b) Vitronectin receptor (VNR); platelet glycoprotein IIbIIIa (GPIIbIIIa); disialyl-lacto-N-tetraose (DSLNT).

Table 5. Reactivity of scFv clones against EGFR. Comparative results between an ELISA method with purified soluble antigen and cytometric analysis of cell lines.

	CLONES	CYTOMETRIC ANALYSIS OF TUMOR CELL LINES ^(a)				ELISA (O.D.)	
		Positive	WM164	A431	MDAAMB231		
5	7H1		1.5	112.9	16.4	2.6	1.2
	4B2		1.2	5.3	4.2	0.6	2
	10D2		1.5	145.3	36.3	4.8	2
	12D2		1.8	129.5	29.3	5.7	2
	5E2		1.4	2.5	7.1	0.5	1.8
	8E2		1.5	134.5	47.7	5.1	1.9
	5F2		1.3	146.3	40.6	5.7	1.9
	11H2		1.9	152.2	25.3	2	1.9
	1B3		0.6	105.1	36.4	5.2	>2
	4B3		0.5	78	15.8	2.3	2
10	3D3		1.2	94.3	25.1	4.8	1.9
	5D3		0.5	112	22.2	5.5	>2
	4F3		0.4	110.3	32.3	6.2	>2
	4G3		0.4	76.5	20.4	2	>2
	1E3		0.4	118.3	33.8	5.1	2
15	3H3		0.6	76.5	33.7	4.2	>2
	Negative						
	5F1		2.4	2.3	3.6	1.8	0.2
	7G1		1.4	10.2	4	2.8	0.2
	1H1		0.5	5	4	0.75	0.2

Table 5: continuation

Controls ^(b)						
5	HEN	0.4	4.1	3.7	1	0.2
	P1	0.6	85.5	21.3	2.5	1.9

a) Three EGFR-bearing cell lines (A431, MDAAMB231 and HT29) and one
10 non-expressing cell line (WM164) were used to assay the ability of scFv to
bind to tumor cells lines by cytometric analysis as described.

b) Vector without fragment (HEN) and scFv fragment from 425 mAb (P1) were
used as negative and positive controls, respectively.

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SEQUENCE LISTING

5 (1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Merck Patent GmbH
- (B) STREET: Frankfurter Str. 255
- (C) CITY: Darmstadt
- (E) COUNTRY: Germany
- (F) POSTAL CODE (ZIP): 64271
- (G) TELEPHONE: 49-6151-727022
- (H) TELEFAX: 49-6151-727191

10

(ii) TITLE OF INVENTION: Anti-EGFR Single-Chain Fvs and Anti-EGFR Antibodies

(iii) NUMBER OF SEQUENCES: 32

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

15

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 327 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

25

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
- (B) STRAIN: Balb/c
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: Lymph node

30

(vii) IMMEDIATE SOURCE:

- (B) CLONE: L2 11C (light chain)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..327

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

35

GAC ATT GAG CTC ACC CAG TCT CCA GCC TCC CTG GCT GCA TCT GTG GGA
Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Val Gly

1

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48

	GAA ACT GTC ACC ATC ACA TGT CGA GCA AGT GAG AAC ATT TAC TAT AGT	96
	Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Tyr Ser	
	20 25 30	
	TTA GCA TGG TAT CAG CAG AAG CAA GGG AAA TCT CCT CAG CTC CTG ATC	144
5	Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile	
	35 40 45	
	TAT AGT GCA AGC GCC TTG GAA GAT GGT GTC CCA TCG AGG TTC AGT GGC	192
	Tyr Ser Ala Ser Ala Leu Glu Asp Gly Val Pro Ser Arg Phe Ser Gly	
	50 55 60	
10	AGT GGA TCT GGG ACA CAG TAT TCT TTA AAG ATC AAC AAC ATG CAG CCT	240
	Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Asn Met Gln Pro	
	65 70 75 80	
	GAA GAT ACC GCT ACT TAC TTC TGT AAA CAG ACT TAT GAC GTT CCG TGG	288
	Glu Asp Thr Ala Thr Tyr Phe Cys Lys Gln Thr Tyr Asp Val Pro Trp	
	85 90 95	
	ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA AAA CGG GCG	327
	Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala	
	100 105	

15 (2) INFORMATION FOR SEQ ID NO: 2:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 109 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

	Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Val Gly	
	1 5 10 15	
	Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Tyr Ser	
	20 25 30	
25	Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile	
	35 40 45	
	Tyr Ser Ala Ser Ala Leu Glu Asp Gly Val Pro Ser Arg Phe Ser Gly	
	50 55 60	
	Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Asn Met Gln Pro	
	65 70 75 80	
30	Glu Asp Thr Ala Thr Tyr Phe Cys Lys Gln Thr Tyr Asp Val Pro Trp	
	85 90 95	
	Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala	
	100 105	

(2) INFORMATION FOR SEQ ID NO: 3:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 357 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

5

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
- (B) STRAIN: Balb/c
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: Lymph Node

(vii) IMMEDIATE SOURCE:

- (B) CLONE: L2 11C (heavy chain)

10

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CAG	G TG	C AA	C TG	C AG	G AG	T CA	G G G	C CT	G AG	C CT	G G G	G CT		48	
Gln	Val	Gln	Leu	Gln	Glu	Ser	Gly	Pro	Glu	Leu	Val	Arg	Pro	Gly	Ala
110										115					125

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TCA	G TG	A AG	A TG	T CC	T GC	A AG	G CT	T CA	G G C	T AT	A CC	T TC	A CT	A CC	T AC		96
Ser	Val	Lys	Met	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Thr	Tyr		
										130		135			140		

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TGG	A TA	C AC	T GG	A TG	A AA	C AG	A GG	C CT	G GA	C AA	G GC	C TT	C AG	T GG	A TT		144
Trp	Ile	His	Trp	Met	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Gln	Trp	Ile		
										145		150			155		

GGC	A TG	A TT	G AT	C CCT	T CC	A AT	G GT	A CT	G AA	A CT	A GG	T TA	A AT	C AG	A AT	T TC		192
Gly	Met	Ile	Asp	Pro	Ser	Asn	Ser	Glu	Thr	Arg	Leu	Asn	Gln	Asn	Phe			
										160		165			170			

AGG	G AC	A AG	G CC	A CA	T TG	A GT	G TA	G AC	A AA	T CC	A AT	A AA	G CC	T AC		240
Arg	Asp	Lys	Ala	Thr	Leu	Ser	Val	Asp	Lys	Ser	Ser	Asn	Lys	Ala	Tyr	
										175		180			185	

25

ATG	C AG	C TC	A GC	A GC	C TG	A CA	T CT	G AG	G AC	T CT	G CA	A TC	T AT	T AC	T G T		288
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Ile	Tyr	Tyr	Cys		
										190		195			200		

GCA	A GA	T GG	G AC	T AC	GGT	A GT	G GC	C AC	TT T	G AC	T AC	T GG	G GC	C AA	G G G		336
Ala	Arg	Trp	Asp	Tyr	Gly	Ser	Gly	His	Phe	Asp	Tyr	Trp	Gly	Gln	Gly		
										210		215			220		

30

ACC	A CG	G TC	A CC	G TC	T CC	T CA										357
Thr	Thr	Val	Thr	Val	Ser	Ser										
										225						

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 119 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Arg Pro Gly Ala
 1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr
 20 25 30

Trp Ile His Trp Met Lys Gln Arg Pro Gly Gln Gly Leu Gln Trp Ile
 5 35 40 45

Gly Met Ile Asp Pro Ser Asn Ser Glu Thr Arg Leu Asn Gln Asn Phe
 50 55 60

Arg Asp Lys Ala Thr Leu Ser Val Asp Lys Ser Ser Asn Lys Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys
 10 85 90 95

Ala Arg Trp Asp Tyr Gly Ser Gly His Phe Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Thr Val Thr Val Ser Ser
 115

(2) INFORMATION FOR SEQ ID NO: 5:

- 15 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 339 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- 20 (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- 25 (vi) ORIGINAL SOURCE:
 (A) ORGANISM: mouse
 (B) STRAIN: Balb/c
 (D) DEVELOPMENTAL STAGE: adult
 (F) TISSUE TYPE: Lymph node
- (vii) IMMEDIATE SOURCE:
 (B) CLONE: L2 12B (light chain)
- (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..339

30

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG 48
 Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 120 125 130 135

35 CAG AGG GCC ACC ATC TCC TGC AGA GCC AGC GAA AGT GTT GAT AAT TTT 96
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
 140 145 150

	GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC	144
	Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro	
	155 160 165	
	 AAA CTC CTC ATC TAT GGT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC	192
	Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala	
	170 175 180	
5	 AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT	240
	Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His	
	185 190 195	
	 CCT CTG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG	288
	Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys	
	200 205 210 215	
10	 GAG GTT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAA ATA AAA CGG	336
	Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg	
	220 225 230	
	 GCG	339
	Ala	

(2) INFORMATION FOR SEQ ID NO: 6:

(2) INFORMATION FOR SEO ID NO: 7:

- 35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 357 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

5 (v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: mouse

(B) STRAIN: Balb/c

(D) DEVELOPMENTAL STAGE: adult

(F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:

10 (B) CLONE: L2 12B (heavy chain)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..357

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

15	CAG GTG CAG CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGG GCT Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala 115 120 125	48
	TTA GTG AAG ATA TCC TGC AAG GCT TCT GGT TAC ACC TTC ACC AGC TAC Leu Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr 130 135 140 145	96
20	TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT GAG TGG ATC Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile 150 155 160	144
	GGA GAG ATT GAT CCT TCT GAT AGT TAT ACT AAC TAC AAT CAA AAG TTC Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe 165 170 175	192
25	AAG GGC AAG GCC ACA TTG ACT GTA GAC AAA TCC TCC AAC ACA GCC TAC Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr 180 185 190	240
	ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys 195 200 205	288
	GCA AGA TCG GAC TAC GGT AGT AGC CAC TTT GAC TAC TGG GGC CAA GGG Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly 210 215 220 225	336
30	ACC ACG GTC ACC GTC TCC TCA Thr Thr Val Thr Val Ser Ser 230	357

(2) INFORMATION FOR SEQ ID NO: 8:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 119 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

Leu Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 5 20 25 30

Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
 50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
 100 105 110

Thr Thr Val Thr Val Ser Ser
 115

(2) INFORMATION FOR SEQ ID NO: 9:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 339 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

20 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

25 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
- (B) STRAIN: Balb/c
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:

- (B) CLONE: L3 11D (light chain)

30 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..339

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG 48
 Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 120 125 130 135

35 CAG AGG GCC ACC ATC TCC TGC CGA GCC AGC GAA AGT GTT GAT AAT TTT 96
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
 140 145 150

	GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro 155 160 165	144
	AAA CTC CTC ATC TAT GGT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala 170 175 180	192
5	AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His 185 190 195	240
	CCT TTG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys 200 205 210 215	288
10	GAG GTT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA CGG Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg 220 225 230	336
	GCG Ala	339

15 (2) INFORMATION FOR SEQ ID NO: 10:

	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 113 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
20	(ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
	Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly 1 5 10 15
	Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe 20 25 30
	Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro 25 35 40 45
25	Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala 50 55 60
	Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His 65 70 75 80
30	Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys 85 90 95
	Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg 100 105 110
	Ala

(2) INFORMATION FOR SEQ ID NO: 11:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 357 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5

- (ii) MOLECULE TYPE: cDNA

- (iii) HYPOTHETICAL: NO

- (iv) ANTI-SENSE: NO

- (v) FRAGMENT TYPE: N-terminal

10

- (vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
 (B) STRAIN: Balb/c
 (D) DEVELOPMENTAL STAGE: adult
 (F) TISSUE TYPE: Lymph node

- (vii) IMMEDIATE SOURCE:

- (B) CLONE: L3 11D (heavy chain)

15

- (ix) FEATURE:

- (A) NAME/KEY: CDS
 (B) LOCATION: 1..357

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

GAG	GTG	CAG	CTG	CAG	CAG	TCA	GGG	GCT	GAG	CTT	GTG	AAG	CCT	GGG	GCT	48
Glu	Val	Gln	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Lys	Pro	Gly	Ala	
115						120						125				
TCA	GTG	AAG	CTG	TCC	TGC	AAG	GCT	TCT	GGC	TAC	ACC	TTC	ACC	AGC	TAC	96
Ser	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	
130					135				140				145			
TGG	ATG	CAC	TGG	GTG	AAG	CAG	AGG	CCT	GGA	CAA	GGC	CTT	GAG	TGG	ATC	144
Trp	Met	His	Trp	Val	Lys	Gln	Arg	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Ile	
					150				155				160			
GGA	GAG	ATT	GAT	CCT	TCT	GAT	AGT	TAT	ACT	AAC	TAC	AAT	CAA	AAG	TTC	192
Gly	Glu	Ile	Asp	Pro	Ser	Asp	Ser	Tyr	Thr	Asn	Tyr	Asn	Gln	Lys	Phe	
					165				170			175				
AAG	GGC	AAG	GCC	ACA	TTG	ACT	GTA	GAC	AAA	TCC	TCC	AGC	ACA	GCC	TAC	240
Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr	
					180			185			190					
ATG	CAG	CTC	AGC	AGC	CTG	ACA	TCT	GAG	GAC	TCT	GCG	GTC	TAT	TAC	TGT	288
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys	
					195			200			205					
GCA	AGA	TCG	GAC	TAC	GGT	AGT	AGC	CAC	TTT	GAC	TAC	TGG	GGC	CAA	GGG	336
Ala	Arg	Ser	Asp	Tyr	Gly	Ser	Ser	His	Phe	Asp	Tyr	Trp	Gly	Gln	Gly	
					210			215			220			225		
ACC	ACG	GTC	ACC	GTC	TCC	TCA										357
Thr	Thr	Val	Thr	Val	Ser	Ser										
					230											

35

(2) INFORMATION FOR SEQ ID NO: 12:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 119 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

10 Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

15 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
100 105 110

Thr Thr Val Thr Val Ser Ser
115

20 (2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 327 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
30 (D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: Lymph node
(vii) IMMEDIATE SOURCE:
(B) CLONE: S4 2D (light chain)
(ix) FEATURE:
(A) NAME/KEY: CDS
35 (B) LOCATION: 1..327

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

	GAC ATT GAG CTC ACC CAG TCT CCA ACC ACC ATG GCT GCA TCT CCC GGG Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Met Ala Ala Ser Pro Gly 120 125 130 135	48
5	GAG AAG ATC ACT ATC ACC TGC AGT GCC AGC TCA AGT ATA AGT TCC AAT Glu Lys Ile Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Ser Asn 140 145 150	96
	TAC TTG CAT TGG TAT CAG CAG AAG CCA GGA TTC TCC CCT AAA CTC TTG Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Phe Ser Pro Lys Leu Leu 155 160 165	144
10	ATT TAT AGG ACA TCC AAT CTG GCT TCT GGA GTC CCA GCT CGC TTC AGT Ile Tyr Arg Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser 170 175 180	192
	GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC ACA ATT GGC ACC ATG GAG Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Gly Thr Met Glu 185 190 195	240
15	GCT GAA GAT GTT GCC ACT TAC TAC TGC CAG CAG GGT AGT AGT ATA CCA Ala Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ser Ile Pro 200 205 210 215	288
	CGC ACG TTC GGA GGG GGC ACC AAG CTG GAA ATC AAA CGG Arg Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Arg. 220 225	327

(2) INFORMATION FOR SEQ ID NO: 14:

20	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 109 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear
	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
25	Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Met Ala Ala Ser Pro Gly 1 5 10 15
	Glu Lys Ile Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Ser Asn 20 25 30
	Tyr Leu His Trp Tyr Gln Lys Pro Gly Phe Ser Pro Lys Leu Leu 35 40 45
30	Ile Tyr Arg Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser 50 55 60
	Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Gly Thr Met Glu 65 70 75 80
	Ala Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ser Ile Pro 85 90 95
35	Arg Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Arg 100 105

(2) INFORMATION FOR SEQ ID NO: 15:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 354 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

10 (vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
- (B) STRAIN: Balb/c
- (D) DEVELOPMENTAL STAGE: adult
- (F) TISSUE TYPE: Lymph node

(vii) IMMEDIATE SOURCE:

- (B) CLONE: S4 2D (heavy chain)

15 (ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..354

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

	GAG GTC AAG CTG CAG CAG TCA GGA CCT GAG CTG GTA AAG CCT GGG GCT	48
	Glu Val Lys Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala	
20	110 115 120 125	
	TCA GTG AAG ATG TCC TGC AAG GCT TCT GGA TAC GCA TTC ATA AGT TTT	96
	Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ile Ser Phe	
	130 135 140	
	GTT ATG CAC TGG GTG AAG CAG AAG CCT GGG CAG GGC CTT GAG TGG ATT	144
	Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile	
	145 150 155	
25	GGA TTT ATT AAT CCT TAC AAT GAT GGT ACT AAG TAC AAT GAG AAG TTC	192
	Gly Phe Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe	
	160 165 170	
	AAA GAC AAG GCC ACA CTG ACT TCA GAC AAA TCC TCC AGC ACA GCC TAC	240
	Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr	
	175 180 185	
30	ATG GAG CTC AGC AGC CTG ACC TCT GAG GAC TCT GCG GTC TAT TAC TGT	288
	Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys	
	190 195 200 205	
	GCA AGT GGG GAT TAC GAC AGG GCT ATG GAC TAC TGG GGC CAA GGG ACC	336
	Ala Ser Gly Asp Tyr Asp Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr	
	210 215 220	
35	ACG GTC ACC GTC TCC TCA	354
	Thr Val Thr Val Ser Ser	
	225	

(2) INFORMATION FOR SEQ ID NO: 16:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 118 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

Glu Val Lys Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ile Ser Phe
20 25 30

10 Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Phe Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

15 Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Ser Gly Asp Tyr Asp Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Val Thr Val Ser Ser
115

20 (2) INFORMATION FOR SEQ ID NO: 17:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 717 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

25 (ii) MOLECULE TYPE: cDNA
(iii) HYPOTHETICAL: NO
(iv) ANTI-SENSE: NO
(v) FRAGMENT TYPE: N-terminal
(vi) ORIGINAL SOURCE:
(A) ORGANISM: mouse
(B) STRAIN: Balb/c
(D) DEVELOPMENTAL STAGE: adult
(F) TISSUE TYPE: splenocytes
(vii) IMMEDIATE SOURCE:
(B) CLONE: 4 B 2
(ix) FEATURE:
30 (A) NAME/KEY: CDS
(B) LOCATION: 1..717

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

	GAG GTG AAG CTG CAG GAG TCT GGG GGA GAC TTA GTG AAG CCT GGA GGG Glu Val Lys Leu Gln Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly 120 125 130	48
5	TCC CTG AAA CTC TCC TGT GCA GCC TCT GGA TTC ACT TTC AGT AGC TAT Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr 135 140 145 150	96
	GGC ATG TCT TGG GTT CGG CAG ACT CCA GAC AAG AGG CTG GAG TCT GTC Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Ser Val 155 160 165	144
10	GCA ACC ATT AGT AGT GGT GGT GCT TAC ATC TAC TAT CCA GAC AGT GTG Ala Thr Ile Ser Ser Gly Gly Ala Tyr Ile Tyr Tyr Pro Asp Ser Val 170 175 180	192
	AAG GGG CGA TTC ACC ATC TCC AGA GAC AAT GCC AAG AAC ACC CTG TAC Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr 185 190 195	240
	CTG CAA ATG AGC AGT CTG AAG TCT GAG GAC ACA GCC ATG TAT TAC TGT Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys 200 205 210	288
15	GCA AGA CTT GAA ACC GGG GAC TAT GCT TTG GAC TAC TGG GGC CAA GGG Ala Arg Leu Glu Thr Gly Asp Tyr Ala Leu Asp Tyr Trp Gly Gln Gly 215 220 225 230	336
	ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GCC GGT GGT GGG Thr Thr Val Thr Val Ser Ser Gly Gly Ser Gly Gly Ser Gly Gly 235 240 245	384
20	TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT Ser Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser 250 255 260	432
	TTG GCT GTC TCT CTA GGG CAG AGG GCC ACC ATA TTC TGC AAG GAC AGC Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Phe Cys Lys Asp Ser 265 270 275	480
25	CAA AGT GTT GAT TAT GAT GGT GAT AGT TAT ATG AAC TGG TAC CAA CAG Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln 280 285 290	528
	AAA CCA GGA CAG CCA CCC AAA CTC CTC ATC TAT GCT CGA TCC AAT CTA Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Arg Ser Asn Leu 295 300 305 310	576
	GAA TCT GGG GTC CCT GCC AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp 315 320 325	624
30	TTC AGC CTC AAC ATC CAT CCT GTG GAG GAG GAT GAT ATT GCA ATG TAT Phe Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr 330 335 340	672
	TTC TGT CAG CAA AGT AGG AAG GTT CCG TGG TCG TTC GGT GGA GGG Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Ser Phe Gly Gly Gly 345 350 355	717

(2) INFORMATION FOR SEQ ID NO: 18:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 239 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

5 (ii) MOLECULE TYPE: protein
(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

Glu Val Lys Leu Gln Glu Ser Gly Gly Asp Leu Val Lys Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 Gly Met Ser Trp Val Arg Gln Thr Pro Asp Lys Arg Leu Glu Ser Val
 35 40 45
 Ala Thr Ile Ser Ser Gly Gly Ala Tyr Ile Tyr Tyr Pro Asp Ser Val
 50 55 60
 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Tyr Tyr Cys
 85 90 95
 Ala Arg Leu Glu Thr Gly Asp Tyr Ala Leu Asp Tyr Trp Gly Gln Gly
 100 105 110
 Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly Gly Gly
 115 120 125
 Ser Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser
 130 135 140
 Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Phe Cys Lys Asp Ser
 145 150 155 160
 Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln
 165 170 175
 Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Arg Ser Asn Leu
 180 185 190
 Glu Ser Gly Val Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp
 195 200 205
 Phe Ser Leu Asn Ile His Pro Val Glu Glu Asp Asp Ile Ala Met Tyr
 210 215 220
 Phe Cys Gln Gln Ser Arg Lys Val Pro Trp Ser Phe Gly Gly Gly
 225 230 235

(2) INFORMATION FOR SEO ID NO: 19:

- (i) SEQUENCE CHARACTERISTICS:

 - (A) LENGTH: 732 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:

(A) ORGANISM: mouse

(B) STRAIN: Balb/c

(F) TISSUE TYPE: splenocytes

5

(vii) IMMEDIATE SOURCE:

(B) CLONE: 10 D 2 (single-chain Fv, heavy and light chain plus linker)

(ix) FEATURE:

(A) NAME/KEY: CDS

(B) LOCATION: 1..732

10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

GAG GTG CAG CTG CAG CAG TCT GGG GCT GAA CTG GTG AAG CCT GGG GCT Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala	48
240 245 250 255	

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His	96
15 260 265 270	

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile	144
275 280 285	

GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe	192
20 290 295 300	

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Thr Ala Tyr	240
305 310 315	

ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys	288
320 325 330 335	

25 GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly	336
340 345 350	

CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly	384
355 360 365	

30 GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA Gly Gly Ser Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro	432
370 375 380	

GCA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser	480
385 390 395	

35 GCC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAA CCA GGA Ala Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Pro Gly	528
400 405 410 415	

60

	TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA	576
	Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly	
	420 425 430	
	GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC	624
	Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu	
	435 440 445	
5	ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG	672
	Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln	
	450 455 460	
	CAG TGG AGT AGT TAC CCA CCC ATG TAC ACG TTC GGA GGG GGG ACC AAG	720
	Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys	
	465 470 475	
10	CTG GAA ATA AAA	732
	Leu Glu Ile Lys	
	480	

(2) INFORMATION FOR SEQ ID NO: 20:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 244 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

	Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala	
	1 5 10 15	
20	Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His	
	20 25 30	
	Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile	
	35 40 45	
	Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe	
	50 55 60	
25	Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr	
	65 70 75 80	
	Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys	
	85 90 95	
	Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly	
	100 105 110	
30	Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly	
	115 120 125	
	Gly Gly Ser Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro	
	130 135 140	
	Ala Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser	
	145 150 155 160	
35	Ala Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Pro Gly	
	165 170 175	

Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
 180 185 190
 Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
 195 200 205
 Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
 210 215 220
 5 Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
 225 230 235 240
 Leu Glu Ile Lys

(2) INFORMATION FOR SEQ ID NO: 21:

10

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 732 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

15

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
- (B) STRAIN: Balb/c
- (F) TISSUE TYPE: splenocytes

20

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 3 D 3 (single-chain Fv, heavy and light chain plus linker)

25

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION:1..732

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

GAG GTC CAA CTG CAG CAG TCA GGG GCT GAA CTG GTG AAG CCT GGG GCT	48
Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala	
245 250 255 260	

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC	96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His	
265 270 275	

30

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC	144
Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile	
280 285 290	

35

GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA ATC	192
Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Ile	
295 300 305	

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC	240
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr	
310 315 320	

	ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys 325 330 335 340	288
5	GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly 345 350 355	336
	CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly 360 365 370	384
	GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA Gly Gly Ser Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro 375 380 385	432
10	ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser 390 395 400	480
	GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG ACA GGA Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly 405 410 415 420	528
15	TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly 425 430 435	576
	GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu 440 445 450	624
20	ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln 455 460 465	672
	CAG TGG AGT AGT TAC CCA CCC ATG TAG ACG TTC GGA GGG GGG ACC AAG Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys 470 475 480	720
25	CTG GAA ATA AAA Leu Glu Ile Lys 485	732
	(2) INFORMATION FOR SEQ ID NO: 22:	
	(i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 244 amino acids (B) TYPE: amino acid (D) TOPOLOGY: linear	
30	(ii) MOLECULE TYPE: protein (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:	
	Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala 1 5 10 15	
	Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His 20 25 30	
35	Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile 35 40 45	

Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Ile
 50 55 60
 Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 5 85 90 95
 Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
 100 105 110
 Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly
 115 120 125
 Gly Gly Ser Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
 10 130 135 140
 Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
 145 150 155 160
 Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
 165 170 175
 Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
 15 180 185 190
 Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
 195 200 205
 Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
 210 215 220
 Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
 20 225 230 235 240
 Leu Glu Ile Lys

(2) INFORMATION FOR SEQ ID NO: 23:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 738 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: mouse
 - (B) STRAIN: Balb/c
 - (F) TISSUE TYPE: splenocytes
- (vii) IMMEDIATE SOURCE:
 - (B) CLONE: 1 E 3 (single-chain Fv, heavy and light chain plus linker)

(ix) FEATURE:

(A) NAME/KEY: CDS
 (B) LOCATION: 1..738

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

	GAG GTG CAG CTG CAG CAG TCT GGG GCT GAA CTG GTG AAG CCT GGG GCT	48
5	Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala	
	245 250 255 260	
	TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC	96
	Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His	
	265 270 275	
10	TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC	144
	Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile	
	280 285 290	
	GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC	192
	Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe	
	295 300 305	
	AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCT TAC	240
	Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr	
	310 315 320	
15	ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT	288
	Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys	
	325 330 335 340	
	GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC	336
	Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly	
	345 350 355	
20	CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT	384
	Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly	
	360 365 370	
	GGT GGG TCG GGT GGC GGC GGA TCT GGA TCT GAC ATT GAG CTC ACC CAG	432
	Gly Gly Ser Gly Gly Ser Gly Ser Asp Ile Glu Leu Thr Gln	
	375 380 385	
25	TCT CCA ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC	480
	Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr	
	390 395 400	
	TGC AGT GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG	528
	Cys Ser Asp Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys	
	405 410 415 420	
30	CCA GGA TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT	576
	Pro Gly Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala	
	425 430 435	
	TCT GGA GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC	624
	Ser Gly Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr	
	440 445 450	
35	TCT CTC ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC	672
	Ser Leu Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr	
	455 460 465	
	TGC CAG CAG TGG AGT AGT TAC CCA CCC ATG TAC ACC TTC GGA GGG GGG	720
	Cys Gln Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly	
	470 475 480	

ACC AAG CTG GAA ATA AAA
 Thr Lys Leu Glu Ile Lys
 485 490

738

(2) INFORMATION FOR SEQ ID NO: 24:

5

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 246 amino acids
 - (B) TYPE: amino acid
 - (D) TOPOLOGY: linear

- (ii) MOLECULE TYPE: protein
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

10	Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala	1	5	10	15
	Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His	20	25	30	
	Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile	35	40	45	
15	Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe	50	55	60	
	Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr	65	70	75	80
	Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys	85	90	95	
20	Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly	100	105	110	
	Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly	115	120	125	
	Gly Gly Ser Gly Gly Ser Gly Ser Asp Ile Glu Leu Thr Gln	130	135	140	
25	Ser Pro Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr	145	150	155	160
	Cys Ser Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys	165	170	175	
	Pro Gly Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala	180	185	190	
30	Ser Gly Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr	195	200	205	
	Ser Leu Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr	210	215	220	
	Cys Gln Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly	225	230	235	240
35	Thr Lys Leu Glu Ile Lys	245			

(2) INFORMATION FOR SEQ ID NO: 25:

- (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 726 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

5

- (ii) MOLECULE TYPE: cDNA
 (iii) HYPOTHETICAL: NO
 (iv) ANTI-SENSE: NO
 (v) FRAGMENT TYPE: N-terminal

10

- (vi) ORIGINAL SOURCE:
 (A) ORGANISM: mouse
 (B) STRAIN: Balb/c
 (F) TISSUE TYPE: splenocytes
 (vii) IMMEDIATE SOURCE:
 (B) CLONE: 5 F 1 (single-chain Fv, heavy, light chain,
 linker)
 (ix) FEATURE:
 (A) NAME/KEY: CDS
 (B) LOCATION: 1..726
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

20	CAG GTG AAA CTG CAG GAG TCT GGG GCT GAA CTG GTG AAG CCT GGG GCT Gln Val Lys Leu Gln Glu Ser Gly Ala Glu Leu Val Lys Pro Gly Ala 250 255 260	48
	TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His 265 270 275	96
25	TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile 280 285 290	144
	GGA GAG ATT AAT CCC AGA ACG GCG CCT ACT AAC TAC AAT GAG AAA TTC Gly Glu Ile Asn Pro Arg Thr Ala Pro Thr Asn Tyr Asn Glu Lys Phe 295 300 305 310	192
30	AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr 315 320 325	240
	ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys 330 335 340	288
	GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly 345 350 355	336
35	CAA GGG ACA ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly 360 365 370	384

	GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA	432
	Gly Gly Ser Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro	
375	380	385
	390	
	ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT	480
	Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser	
5	395	400
	405	
	GAC AGC TCA AGT GTA AGT TAC ACG TAC TGG TAC CAG CAG AAG ACA GGA	528
	Asp Ser Ser Val Ser Tyr Thr Tyr Trp Tyr Gln Gln Lys Thr Gly	
	410	415
	420	
	TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA	576
	Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly	
	425	430
	435	
10	GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC	624
	Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu	
	440	445
	450	
	ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG	672
	Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln	
	455	460
	465	
	470	
15	CAG TGG AGT AGT TAC CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAA	720
	Gln Trp Ser Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu	
	475	480
	485	
	ATA AAA	726
	Ile Lys	

20 (2) INFORMATION FOR SEQ ID NO: 26:

- (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 242 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

) MOLECULE TYPE: protein
)) SEQUENCE DESCRIPTION: SEQ ID NO: 26;

25 Gln Val Lys Leu Gln Glu Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
 1 5 10 15
 Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
 20 25 30
 Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 30 Gly Glu Ile Asn Pro Arg Thr Ala Pro Thr Asn Tyr Asn Glu Lys Phe
 50 55 60
 Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95
 35 Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
 115 120 125
 Gly Gly Ser Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
 130 135 140
 Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
 145 150 155 160
 Asp Ser Ser Ser Val Ser Tyr Thr Tyr Trp Tyr Gln Gln Lys Thr Gly
 165 170 175
 Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
 180 185 190
 Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
 195 200 205
 Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
 210 215 220
 Gln Trp Ser Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu
 225 230 235 240
 Ile Lys

15

(2) INFORMATION FOR SEQ ID NO: 27:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 726 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: cDNA
- (iii) HYPOTHETICAL: NO
- (iv) ANTI-SENSE: NO
- (v) FRAGMENT TYPE: N-terminal
- (vi) ORIGINAL SOURCE:
 - (A) ORGANISM: mouse
 - (B) STRAIN: Balb/c
 - (F) TISSUE TYPE: splenocytes
- (vii) IMMEDIATE SOURCE:
 - (A) LIBRARY: 7 G 1 (single-chain Fv, heavy, light chain, linker)
- (ix) FEATURE:
 - (A) NAME/KEY: CDS
 - (B) LOCATION: 1..726

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

GAG GTC AAG CTG CAG CAG TCA GGG GCT GAA CTG GTG AAG CCT GGG GCT	48
Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala	
245 250 255	
TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC	96
Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His	
260 265 270	

	TTG GAT CAC TGG GTG AAG CAG AGG GGC TGG CAA GGC CTT GAG TGG ATC Leu Asp His Trp Val Lys Gln Arg Gly Trp Gln Gly Leu Glu Trp Ile 275 280 285 290	144
5	GGA CAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC Gly Gln Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe 295 300 305	192
	AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr 310 315 320	240
	ATC GAA CTC AGC AGC CTG ACA TCT GAG GAC TGC TCG GTC TAT TAC TGT Ile Glu Leu Ser Ser Leu Thr Ser Glu Asp Cys Ser Val Tyr Tyr Cys 325 330 335	288
10	GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly 340 345 350	336
	CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly 355 360 365 370	384
15	GGT GGG TCG GGT GGC GGC CGA TCT GAC ATT GAG CTC ACC CAG TCT CCA Gly Gly Ser Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro 375 380 385	432
	ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser 390 395 400	480
20	GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG ACA GGA Asp Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly 405 410 415	528
	TCC TCC CCC AGA CTT CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly 420 425 430	576
25	GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu 435 440 445 450	624
	ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln 455 460 465	672
	CAG TGG AGT AGT TAC CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAA Gln Trp Ser Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu 470 475 480	720
30	ATA AAA Ile Lys	726

(2) INFORMATION FOR SEQ ID NO: 28:

- 35 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 242 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

	Glu	Val	Lys	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Lys	Pro	Gly	Ala
	1				5				10					15		
5	Ser	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	His
					20				25					30		
	Leu	Asp	His	Trp	Val	Lys	Gln	Arg	Gly	Trp	Gln	Gly	Leu	Glu	Trp	Ile
					35			40				45				
	Gly	Gln	Phe	Asn	Pro	Ser	Asn	Gly	Arg	Thr	Asn	Tyr	Asn	Glu	Lys	Phe
					50			55			60					
10	Lys	Ser	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr
					65			70			75			80		
	Ile	Glu	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Cys	Ser	Val	Tyr	Tyr	Cys
					85				90				95			
	Ala	Ser	Arg	Asp	Tyr	Asp	Tyr	Asp	Gly	Arg	Tyr	Phe	Asp	Tyr	Trp	Gly
					100				105			110				
15	Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Ser	Gly	Gly	
					115			120			125					
	Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro
					130			135			140					
	Thr	Ile	Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Ser
					145			150			155			160		
20	Asp	Ser	Ser	Ser	Val	Ser	Tyr	Met	Tyr	Trp	Tyr	Gln	Gln	Lys	Thr	Gly
					165			170			175				175	
	Ser	Ser	Pro	Arg	Leu	Leu	Ile	Tyr	Asp	Thr	Ser	Asn	Leu	Ala	Ser	Gly
					180				185			190				
	Val	Pro	Val	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Ser	Tyr	Ser	Leu
					195			200			205					
25	Thr	Ile	Ser	Arg	Met	Glu	Ala	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln
					210			215			220					
	Gln	Trp	Ser	Ser	Tyr	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu
					225			230			235			240		
	Ile	Lys														

30

(2) INFORMATION FOR SEQ ID NO: 29:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 726 base pairs
 (B) TYPE: nucleic acid
 (C) STRANDEDNESS: single
 (D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

(A) ORGANISM: mouse

5 (B) STRAIN: Balb/c

(D) DEVELOPMENTAL STAGE: adult

(F) TISSUE TYPE: splenocytes

(vii) IMMEDIATE SOURCE:

(B) CLONE: 11 H 1 (single-chain Fv, heavy and light
chain plus linker)

(ix) FEATURE:

10 (A) NAME/KEY: CDS
(B) LOCATION: 1..726

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 29:

GAG	GTC	AAG	CTG	CAG	CAG	TCA	GGG	GCT	GAA	CTG	GTG	AAG	CCT	GGG	GCT		48
Glu	Val	Lys	Leu	Gln	Gln	Ser	Gly	Ala	Glu	Leu	Val	Lys	Pro	Gly	Ala		
245							250					255					
TCA	GTG	AAG	TTG	TCC	TGC	AAG	GCT	TCC	GGC	TAC	ACC	TTC	ACC	AGC	CAC		96
Ser	Val	Lys	Leu	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	His		
260						265					270						
TGG	ATG	CAC	TGG	GTG	AAG	CAG	AGG	GCT	GGA	CAA	GGC	TTG	GAG	TGG	ATC		144
Trp	Met	His	Trp	Val	Lys	Gln	Arg	Ala	Gly	Gln	Gly	Leu	Glu	Trp	Ile		
275						280				285				290			
GGA	GAG	TTT	AAT	CCC	AGC	AAC	GGC	CGT	ACT	AAC	TAC	AAT	GAG	AAA	TTC		192
Gly	Glu	Phe	Asn	Pro	Ser	Asn	Gly	Arg	Thr	Asn	Tyr	Asn	Glu	Lys	Phe		
295						300				305							
AAG	AGC	AAG	GCC	ACA	CTG	ACT	GTA	GAC	AAA	TCC	TCC	AGC	ACA	GCC	TAC		240
Lys	Ser	Lys	Ala	Thr	Leu	Thr	Val	Asp	Lys	Ser	Ser	Ser	Thr	Ala	Tyr		
310						315				320							
ATG	CAA	CTC	AGC	AGC	CTG	ACA	TCT	GAG	GAC	TCT	GCG	GTC	TAT	TAC	TGT		288
Met	Gln	Leu	Ser	Ser	Leu	Thr	Ser	Glu	Asp	Ser	Ala	Val	Tyr	Tyr	Cys		
325						330				335							
GCC	AGT	CGG	GAC	TAT	GAT	TAC	GAC	GGA	CGG	TAC	TTT	GAC	TAC	TGG	GGC		336
Ala	Ser	Arg	Asp	Tyr	Asp	Tyr	Asp	Gly	Arg	Tyr	Phe	Asp	Tyr	Trp	Gly		
340						345				350							
CAA	GGG	ACC	ACG	GTC	ACC	GTC	TCC	TCA	GGT	GGC	GGT	GGC	TCG	GGC	GGT		384
Gln	Gly	Thr	Thr	Val	Thr	Val	Ser	Ser	Gly	Gly	Gly	Gly	Ser	Gly	Gly		
355						360				365				370			
GGT	GGG	TCG	GGT	GGC	GGC	GGA	TCT	GAC	ATT	GAG	CTC	ACC	CAG	TCT	CCA		432
Gly	Gly	Ser	Gly	Gly	Gly	Gly	Ser	Asp	Ile	Glu	Leu	Thr	Gln	Ser	Pro		
375						380				385							
TCA	ATC	ATG	TCT	GCA	TCT	CCA	GGG	GAG	AAG	GTC	ACC	ATG	ACC	TGC	AGT		480
Ser	Ile	Met	Ser	Ala	Ser	Pro	Gly	Glu	Lys	Val	Thr	Met	Thr	Cys	Ser		
390						395				400							
GAC	AGC	TCA	AGT	GTA	AGT	TAC	ATG	TAC	TGG	TAC	CAG	CAG	AAG	ACA	GGA		528
Asp	Ser	Ser	Ser	Val	Ser	Tyr	Met	Tyr	Trp	Tyr	Gln	Gln	Lys	Thr	Gly		
405						410				415							

TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly 420 425 430	576
GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu 435 440 445 450	624
5 ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln 455 460 465	672
CAG TGG AGT AGT TAC CCA CAC ACG TTC GGT GCT GGG ACC AAG CTG GAA Gln Trp Ser Ser Tyr Pro His Thr Phe Gly Ala Gly Thr Lys Leu Glu 470 475 480	720
10 ATA AAA Ile Lys	726

(2) INFORMATION FOR SEQ ID NO: 30:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 242 amino acids
 (B) TYPE: amino acid
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 30:

Glu Val Lys Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala 1 5 10 15
20 Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His 20 25 30
Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile 35 40 45
25 Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe 50 55 60
Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr 65 70 75 80
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys 85 90 95
30 Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly 100 105 110
Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Ser Gly Gly 115 120 125
Gly Gly Ser Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro 130 135 140
Ser Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser 145 150 155 160
35 Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly 165 170 175

Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
 180 185 190
 Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
 195 200 205
 Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
 210 215 220
 5 Gln Trp Ser Ser Tyr Pro His Thr Phe Gly Ala Gly Thr Lys Leu Glu
 225 230 235 240
 Ile Lys

(2) INFORMATION FOR SEQ ID NO: 31:

10

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 732 base pairs
 - (B) TYPE: nucleic acid
 - (C) STRANDEDNESS: single
 - (D) TOPOLOGY: linear

15 (ii) MOLECULE TYPE: cDNA

15

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(v) FRAGMENT TYPE: N-terminal

(vi) ORIGINAL SOURCE:

- (A) ORGANISM: mouse
- (B) STRAIN: Balb/c
- (F) TISSUE TYPE: splenocytes

20

(vii) IMMEDIATE SOURCE:

- (B) CLONE: 1 A 1 (single-chain Fv, heavy and light chain plus linker)

(ix) FEATURE:

- (A) NAME/KEY: CDS
- (B) LOCATION: 1..732

25

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 31:

GAG GTG CAG CTG CAG CAG TCT GGG GCT GAA CTG GTG AAG CCT GGG GCT 48
 Glu Val Gln Leu Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala
 245 250 255

TCA GTG AAG TTG TCC TGC AAG GCT TCC GGC TAC ACC TTC ACC AGC CAC 96
 Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His
 30 260 265 270

TGG ATG CAC TGG GTG AAG CAG AGG GCT GGA CAA GGC CTT GAG TGG ATC 144
 Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile
 275 280 285 290

GGA GAG TTT AAT CCC AGC AAC GGC CGT ACT AAC TAC AAT GAG AAA TTC 192
 Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
 295 300 305

35

AAG AGC AAG GCC ACA CTG ACT GTA GAC AAA TCC TCC AGC ACA GCT TAC 240
 Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 310 315 320

	ATG CAA CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys 325 330 335	288
5	GCC AGT CGG GAC TAT GAT TAC GAC GGA CGG TAC TTT GAC TAC TGG GGC Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly 340 345 350	336
	CAA GGG ACC ACG GTC ACC GTC TCC TCA GGT GGC GGT GGC TCG GGC GGT Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly 355 360 365 370	384
	GGT GGG TCG GGT GGC GGC GGA TCT GAC ATT GAG CTC ACC CAG TCT CCA Gly Gly Ser Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro 375 380 385	432
10	ACA ATC ATG TCT GCA TCT CCA GGG GAG AAG GTC ACC ATG ACC TGC AGT Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser 390 395 400	480
	GAC AGC TCA AGT GTA AGT TAC ATG TAC TGG TAC CAG CAG AAG ACA GGA Asp Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly 405 410 415	528
15	TCC TCC CCC AGA CTC CTG ATT TAT GAC ACA TCC AAC CTG GCT TCT GGA Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly 420 425 430	576
	GTC CCT GTT CGC TTC AGT GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu 435 440 445 450	624
20	ACA ATC AGC CGA ATG GAG GCT GAA GAT GCT GCC ACT TAT TAC TGC CAG Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln 455 460 465	672
	CAG TGG AGT AGT TAC CCA CCC ATG TAC ACG TTC GGA GGG GGG ACA AAG Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Thr Lys 470 475 480	720
25	TTG GAA ATA AAA Leu Glu Ile Lys 485	732

(2) INFORMATION FOR SEQ ID NO: 32:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 244 amino acids
- (B) TYPE: amino acid
- (D) TOPOLOGY: linear

30	(ii) MOLECULE TYPE: protein
	(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 32:

	Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala 1 5 10 15
	Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser His 20 25 30
35	Trp Met His Trp Val Lys Gln Arg Ala Gly Gln Gly Leu Glu Trp Ile 35 40 45

Gly Glu Phe Asn Pro Ser Asn Gly Arg Thr Asn Tyr Asn Glu Lys Phe
 50 55 60

Lys Ser Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 5 85 90 95

Ala Ser Arg Asp Tyr Asp Tyr Asp Gly Arg Tyr Phe Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Thr Val Thr Val Ser Ser Gly Gly Gly Gly Ser Gly Gly
 115 120 125

Gly Gly Ser Gly Gly Gly Ser Asp Ile Glu Leu Thr Gln Ser Pro
 10 130 135 140

Thr Ile Met Ser Ala Ser Pro Gly Glu Lys Val Thr Met Thr Cys Ser
 145 150 155 160

Asp Ser Ser Ser Val Ser Tyr Met Tyr Trp Tyr Gln Gln Lys Thr Gly
 165 170 175

Ser Ser Pro Arg Leu Leu Ile Tyr Asp Thr Ser Asn Leu Ala Ser Gly
 15 180 185 190

Val Pro Val Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu
 195 200 205

Thr Ile Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln
 210 215 220

Gln Trp Ser Ser Tyr Pro Pro Met Tyr Thr Phe Gly Gly Gly Thr Lys
 20 225 230 235 240

Leu Glu Ile Lys

25

30

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Patent Claims

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1. Anti-EGFR single-chain Fvs obtainable from phage-antibody libraries constructed from cells from an immunized mammalian.

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2. Antibody fragment according to Claim 1 obtainable from cells of an immunized mouse.

15

3. Antibody fragment according to Claims 1 or 2 obtainable from cells of

- (i) the lymph node,
- (ii) the spleen, or
- (iii) in vitro immunized cells.

20

4. Antibody fragment according to Claims 1 - 3, wherein the variable regions of the heavy and light chain comprise a DNA and/or an amino acid sequence selected from one of the heavy and light chain sequences given in Sequence Id. Nos. 1- 32.

30

5. Anti-EGFR antibody constructed from DNA sequences deriving from antibody fragments according to Claims 1 - 4 and from DNA sequences deriving from constant regions of human immunoglobulins.

35

6. Antibody according to Claim 5 wherein the heavy constant chain region comprises the amino acid sequence of a human gamma-1 chain, and the light constant chain region comprises the amino acid sequence of a human kappa chain.

5

7. Process for the preparation of an anti-EGFR single-chain Fv according to one of the Claims 1 - 4 comprising the following steps:

- 10 (i) isolating RNA from immunized mammalian cells, preferably mouse cells,
- (ii) synthesizing first-strand cDNA,
- (iii) amplifying the V_H and V_k genes in cDNAs from the immunized cells,
- 15 (iv) cloning said genes together with suitable restriction sites into a phagemid vector,
- (v) transforming prokaryotic cells with the ligation mixtures,
- 20 (vi) screening the phage libraries for phage antibodies directed to EGFR using purified EGFR, and
- (vii) producing the desired single-chain Fv in prokaryotic host cells, preferably E. coli.

25

8. Process for the preparation of a whole anti-EGFR antibody by cloning the DNA coding for the variable regions of anti-EGFR antibody fragments produced according to Claim 7 into at least one eukaryotic expression vector containing genomic DNA which codes for the constant regions of human immunoglobulins, transforming eukaryotic cells with said vector(s) and expressing and isolating the antibody.

35

9. Pharmaceutical composition comprising an anti-EGFR antibody fragment according to one of the Claims 1 - 4 or a whole anti-EGFR antibody according to Claims 5 or 6.

5

10. Use of an anti-EGFR antibody fragment according to one of the Claims 1 - 4, or a whole anti-EGFR antibody according to one of the Claims 5 or 6 for the manufacture of a drug directed to tumors or for
10 the diagnostic location and assessment of tumor growth.

15

20

25

30

35

FIGURE 1 (A)

VH:

scFv	FR 1	CDR 1	FR 2
L3 11D	EVQLQQSGAELVKPGASVKLSCKASGYTFT	SYWMH	WVKQRPGQGLEWIG
L2 12B	QVQLQESGPVELVKPGALVKISCKASGYTFT	SYWMH	WVKQRPGQGLEWIG
L3 10A	QVQLQESGGDLVKPGSLKLSCAA SGFTFS	SYGMS	WVRQTPDKRLESVA
L2 1C	EVKLQQSGAELVRPEAVKLSCKTSGYIFT	NYWIH	WVKQRSGQGLEWIA
L2 8C	QVQLQESGAELVRPGASVKLSCKTSGYIFT	NYWIH	WVKQRSGQGLEWIA
L2 11C	QVQLQESGPVELVRPGASVKMSCKASGYTFT	TYWIH	WMKQRPGQGLQWIG
scFv	CDR 2	FR 3	
L3 11D	EIDPSDSYTNYNQKFKG	KATLTVDKSSSTAYMQLSSLTSEDSAVYYCAR	
L2 12B	EIDPSDSYTNYNQKFKG	KATLTVDKSSNTAYMQLSSLTSEDSAVYYCAR	
L3 10A	TISSGGAYIYYPDVKKG	RFTISRDNAKNTLYLQMSSLKSEDTAMYYCAR	
L2 1C	RIYPNGNGSTYYNEKFKG	KATLTADKSSSTAYMQLSSLKSEDSAVYFCAR	
L2 8C	KDLSWNGSYYNEKFKG	KATLTADKSSSTAYMQLSSLKSEDSAVYFCAR	
L2 11C	MIDPSNSETRLNQNFRD	KATLSVDKSSNKAYMQLSSLTSEDSAIYYCAR	
scFv	CDR 3	FR 4	
L3 11D	SDYGSSHFDY	WGQGTTVTVSS	
L2 12B	SDYGSSHFDY	WGQGTTVTVSS	
L3 10A	LETGDYALDY	WGQGTTVTVSS	
L2 1C	STDSSL PYWYFDV	WGQGTTVTVSS	
L2 8C	STDSSL PYWYFDV	WGQGTTVTVSS	
L2 11C	WDYGSGHFDY	WGQGTTVTVSS	

VK:

scFv	FR 1	CDR 1	FR 2
L3 11D	DIELTQSPASLA VSLGQRATISC	RASESVDFNFGISFMN	WFQQKPGQPPKLLIY
L2 12B	DIELTQSPASLA VSLGQRATISC	RASESVDFNFGISFMN	WFQQKPGQPPKLLIY
L3 10A	DIELTQSPASLA VSLGQRATISC	RASESVEYYGTSLMQ	WFQQKPGQPPKLLIY
L2 1C	DIELTQSPTILSTSPGEKVTVTC	RATLGVS YMH	WYQQKPGSSPKPWYI
L2 8C	DIELTQSPA IMA SASPGEKVTITC	SASSSVSYM H	WFQQKPGTSPKLWYI
L2 11C	DIELTQSPASLA ASVG ETVITC	RASENIYYSLA	WYQQKQGKSPQLLIY
scFv	CDR 2	FR 3	
L3 11D	GASNQGS	GVPARFSGSGSGTDFSLNIHPLEEDDTAMYFC	
L2 12B	GASNQGS	GVPARFSGSGSGTDFSLNIHPLEEDDTAMYFC	
L3 10A	AASNVES	GVPARFSGSGSGTDFSLNIHPVEEDDIAMYFC	
L2 1C	ATSNLAS	GVPARFSGSGSGTSYSLTISRVEAEDAATYYC	
L2 8C	STS NLAS	GVPARFSGSGSGTSYSLTISRMEAEDAATYYC	
L2 11C	SASALED	GVPSRFSGSGSGTQYSLKINNMQPEDTATYFC	
scFv	CDR 3	FR 4	
L3 11D	QQSK EVPLT	FGAGTKLELKRA	
L2 12B	QQSK EVPLT	FGAGTKLELKRA	
L3 10A	QQSRK VPWT	FGGGT KLEIKRA	
L2 1C	QQWISNPPT	FGGGT KLEIKRA	
L2 8C	QQRNSYPHT	FGAGTKLELKRA	
L2 11C	KQTYDVPWT	FGGGT KLEIKRA	

FIGURE 1 (B)

VH:

scFv	FR 1	CDR 1	FR 2
S4 2D	EVKLQQSGPELVKPGASVKMSCKASGYAFI	SFVMH	WVKQKPGQGLEWIG
S4 10H	EVKLQESGGDLVKPGGSLKLSCAA SGFTFS	SYGMS	WVRQTPDKRLESVA
S4 5A	EVKLQESGGDSVKPGGSLKLSCAA SGFTFS	SYGMS	WVRQTPDKRLESVA
S3 12D	EVKLQQSGAELVKPGASVJKLSCASGFNIK	DTYMH	WVKQRPEQGLEWIG
scFv	CDR 2	FR 3	
S4 2D	FINPYNDGTKYNEKFKD	KATLTSKSSSTAYMELSSLTSEDSAVYYCAS	
S4 10H	TISSGGAYIYYPD SVKG	RFTISRDNAKNTLYLQMSSLKSEDTAMYYCAR	
S4 5A	TISSGGAYIYYPD SVKG	RFTISRDNAKNTLYLQMSSLKSEDTAMYYCAR	
S3 12D	RIDPANGNTKYDPKFQD	RASITADTSSNTAYLQLSSLTSEDTAVYYCAS	
scFv	CDR 3	FR 4	
S4 2D	GDYDRAMDY	WGQGTTTVSS	
S4 10H	LETGDYALDY	WGQGTTTVSS	
S4 5A	LETGDYAMDY	WGQGTTTVSS	
S3 12D	DYYGYEAWFAY	WGQGTTTVSS	

VK:

scFv	FR 1	CDR 1	FR 2
S4 2D	DIELTQSPTTMAASPGEKITTC	SASSSISSNYLH	WYQQKPGFSPKLLIY
S4 10H	DIELTQSPASLA VSLGQRATISC	RASESVEYYGTSLMQ	WYQQKPGQAPKLLIY
S4 5A	DIELTQSPASLA VSLGQRATISC	RASESVEYYGTSLMQ	WYQQKPGQPPKLLIY
S3 12D	DIELTQSPASLA VSLGQRATISC	RASESVNDYGISFMN	WYQQKPGQPPKLLIY
scFv	CDR 2	FR 3	
S4 2D	RTSNLAS	GVPARFSGSGSGT SYSLTIGTMEAEDVATYYC	
S4 10H	AASNVES	EVPARFSGSGSGTDFSLNIHPVEEDDIAMYFC	
S4 5A	AASNVES	GVPARFSGSGSGTDFSLNIHPVEEDDIAMYFC	
S3 12D	AASNQGS	GVPARFSGSGSGTDFSLNIHPMEEDDTAMYFC	
scFv	CDR 3	FR 4	
S4 2D	QQGSSIPRT	FGGGT KLEIKRA	
S4 10H	QQSRKVPWT	FGGGT KLEIKRA	
S4 5A	QQSRKVPWT	FGGGT KLEIKRA	
S3 12D	QQSKEVPWT	FGGGT KLEIKRA	

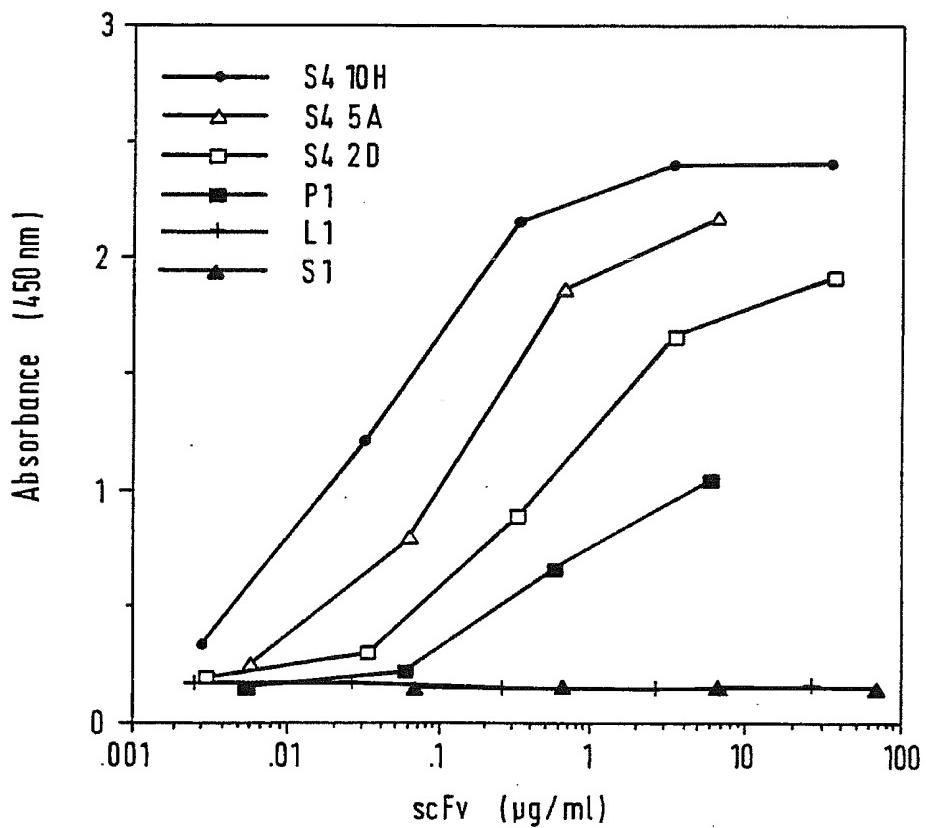
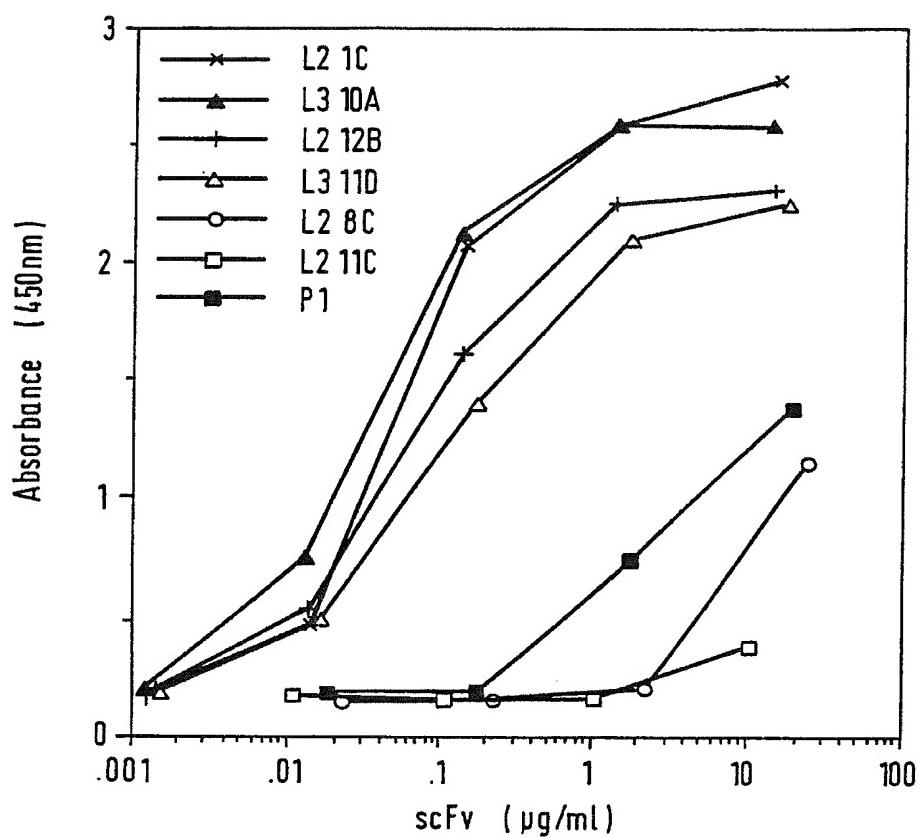
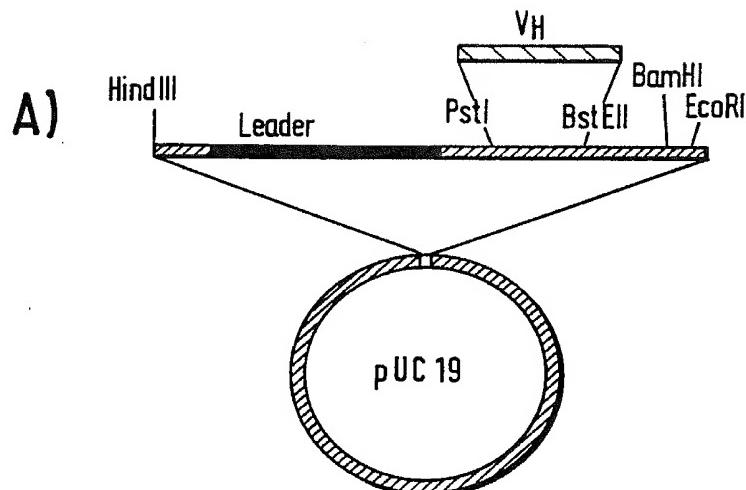
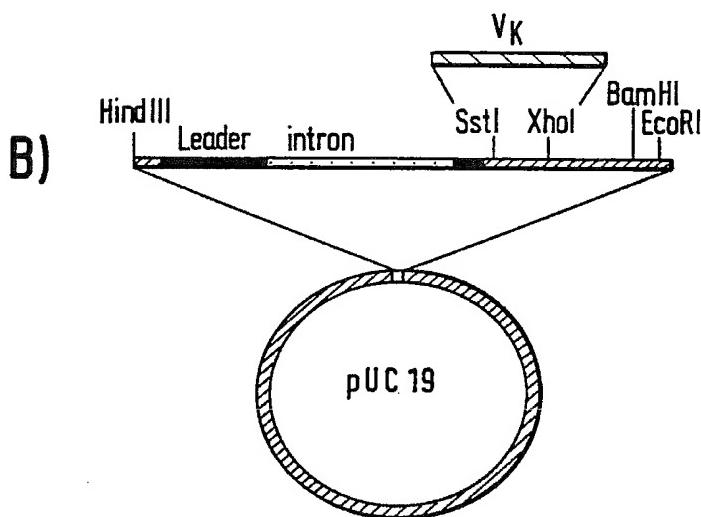


Fig.3



HindIII Leader
 AAGCTTGCCTCCACCATGGAATGGACTGGACCTGGCGCGTGTTTGCTGCTCGCCGTGGCTCCTG
 M D W T W R V F C L L A V A F
 PstI BstEII
 GGGCC CACAGCCAGGTGCAACTGCAGCAGTCGGTGCAAGGGACCACGGTCACCGTCCTCC
 G A H S Q V Q L Q V T V S
 BamHI EcoRI
 TCAGGTGAGTGGATCCGAATTCS



HindIII Leader intron
 AAGCTTGCCTCCACCATGGATGGACCTGTATCATCCTCTTCTTGGTAGCAACAGCTACAGGTA
 M G W S C I I L F L V A T A T
 AGGGGCTCACAGTAGCAGGCTTGAGG TCTGGACATATATGGTGACAATGACATCCACTT
 Leader SstI XbaI
 TGCCCTTCTCTCCACAGGTGTCCACTCCGACATTGAGCTCACCCAGTCCTCAAGAAAGCTC
 G V H S O I E L
 BamHI EcoRI
 GAGCTGAAACGTGAGTA GAATTAAACTTTGCTTCTCAATTGGATCCGAATTCE
 E L K

Fig. 4

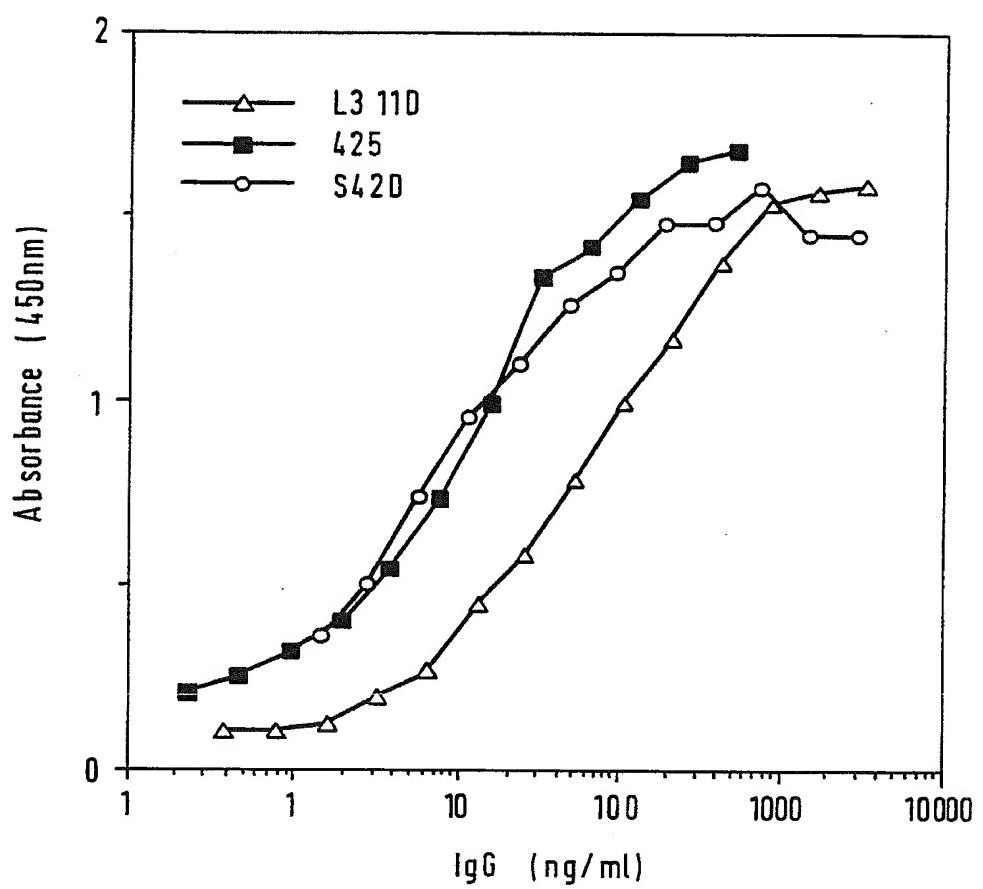


Figure 5.**(A)**

GAC ATT GAG CTC ACC CAG TCT CCA GCC TCC CTG GCT GCA TCT GTG GGA	48
Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Ala Ser Val Gly	
1 5 10 15	
GAA ACT GTC ACC ATC ACA TGT CGA GCA AGT GAG AAC ATT TAC TAT AGT	96
Glu Thr Val Thr Ile Thr Cys Arg Ala Ser Glu Asn Ile Tyr Tyr Ser	
20 25 30	
TTA GCA TGG TAT CAG CAG AAG CAA GGG AAA TCT CCT CAG CTC CTG ATC	144
Leu Ala Trp Tyr Gln Gln Lys Gln Gly Lys Ser Pro Gln Leu Leu Ile	
35 40 45	
TAT AGT GCA AGC GCC TTG GAA GAT GGT GTC CCA TCG AGG TTC AGT GGC	192
Tyr Ser Ala Ser Ala Leu Glu Asp Gly Val Pro Ser Arg Phe Ser Gly	
50 55 60	
AGT GGA TCT GGG ACA CAG TAT TCT TTA AAG ATC AAC AAC ATG CAG CCT	240
Ser Gly Ser Gly Thr Gln Tyr Ser Leu Lys Ile Asn Asn Met Gln Pro	
65 70 75 80	
GAA GAT ACC GCT ACT TAC TTC TGT AAA CAG ACT TAT GAC GTT CCG TGG	288
Glu Asp Thr Ala Thr Tyr Phe Cys Lys Gln Thr Tyr Asp Val Pro Trp	
85 90 95	
ACG TTC GGT GGA GGG ACC AAG CTG GAA ATA AAA CGG GCG	327
Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala	
100 105	

(B):

CAG GTG CAA CTG CAG GAG TCA GGG CCT GAG CTG GTG AGG CCT GGG GCT	48
Gln Val Gln Leu Gln Glu Ser Gly Pro Glu Leu Val Arg Pro Gly Ala	
1 5 10 15	
TCA GTG AAG ATG TCC TGC AAG GCT TCA GGC TAT ACC TTC ACT ACC TAC	96
Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Thr Tyr	
20 25 30	
TGG ATA CAC TGG ATG AAA CAG AGG CCT GGA CAA GGC CTT CAG TGG ATT	144
Trp Ile His Trp Met Lys Gln Arg Pro Gly Gln Gly Leu Gln Trp Ile	
35 40 45	
GGC ATG ATT GAT CCT TCC AAT AGT GAA ACT AGG TTA AAT CAG AAT TTC	192
Gly Met Ile Asp Pro Ser Asn Ser Glu Thr Arg Leu Asn Gln Asn Phe	
50 55 60	
AGG GAC AAG GCC ACA TTG AGT GTA GAC AAA TCC TCC AAT AAA GCC TAC	240
Arg Asp Lys Ala Thr Leu Ser Val Asp Lys Ser Ser Asn Lys Ala Tyr	
65 70 75 80	
ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCA ATC TAT TAC TGT	288
Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr Cys	
85 90 95	
GCA AGA TGG GAC TAC GGT AGT GGC CAC TTT GAC TAC TGG GGC CAA GGG	336
Ala Arg Trp Asp Tyr Gly Ser Gly His Phe Asp Tyr Trp Gly Gln Gly	
100 105 110	
ACC ACG GTC ACC GTC TCC TCA	357
Thr Thr Val Thr Val Ser Ser	
115	

Figure 6. (A)

GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG 48
 Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly
 1 5 10 15

CAG AGG GCC ACC ATC TCC TGC AGA GCC AGC GAA AGT GTT GAT AAT TTT 96
 Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe
 20 25 30

GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC 144
 Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro
 35 40 45

AAA CTC CTC ATC TAT GGT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC 192
 Lys Leu Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala
 50 55 60

AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT 240
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His
 65 70 75 80

CCT CTG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG 288
 Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys
 85 90 95

GAG GTT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAA ATA AAA CGG 336
 Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Ile Lys Arg
 100 105 110

GGC
Ala 339

(B)

CAG GTG CAG CTG CAG GAG TCT GGA CCT GAG CTG GTG AAG CCT GGG GCT 48
 Gln Val Gln Leu Glu Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

TTA GTG AAG ATA TCC TGC AAG GCT TCT GGT TAC ACC TTC ACC AGC TAC 96
 Leu Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT GAG TGG ATC 144
 Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

GGA GAG ATT GAT CCT TCT GAT AGT TAT ACT AAC TAC AAT CAA AAG TTC 192
 Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
 50 55 60

AAG GGC AAG GCC ACA TTG ACT GTA GAC AAA TCC TCC AAC ACA GCC TAC 240
 Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Asn Thr Ala Tyr
 65 70 75 80

ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT 288
 Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

GCA AGA TCG GAC TAC GGT AGT AGC CAC TTT GAC TAC TGG GGC CAA GGG 336
 Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly
 100 105 110

ACC ACG GTC ACC GTC TCC TCA 357
 Thr Thr Val Thr Val Ser Ser
 115

Figure 7: (A)

GAC ATT GAG CTC ACC CAG TCT CCA GCT TCT TTG GCT GTG TCT CTA GGG Asp Ile Glu Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly 1 5 10 15	48
CAG AGG GCC ACC ATC TCC TGC CGA GCC AGC GAA AGT GTT GAT AAT TTT Gln Arg Ala Thr Ile Ser Cys Arg Ala Ser Glu Ser Val Asp Asn Phe 20 25 30	96
GGC ATT AGT TTT ATG AAC TGG TTC CAA CAG AAA CCA GGA CAG CCA CCC Gly Ile Ser Phe Met Asn Trp Phe Gln Gln Lys Pro Gly Gln Pro Pro 35 40 45	144
AAA CTC CTC ATC TAT GGT GCA TCC AAC CAA GGA TCC GGG GTC CCT GCC Lys Leu Ile Tyr Gly Ala Ser Asn Gln Gly Ser Gly Val Pro Ala 50 55 60	192
AGG TTT AGT GGC AGT GGG TCT GGG ACA GAC TTC AGC CTC AAC ATC CAT Arg Phe Ser Gly Ser Gly Thr Asp Phe Ser Leu Asn Ile His 65 70 75 80	240
CCT TTG GAG GAG GAT GAT ACT GCA ATG TAT TTC TGT CAG CAA AGT AAG Pro Leu Glu Glu Asp Asp Thr Ala Met Tyr Phe Cys Gln Gln Ser Lys 85 90 95	288
GAG GTT CCG CTC ACG TTC GGT GCT GGG ACC AAG CTG GAG CTG AAA CGG Glu Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg 100 105 110	336
GCG Ala	339
(B)	
GAG GTG CAG CTG CAG CAG TCA GGG GCT GAG CTT GTG AAG CCT GGG GCT Glu Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Lys Pro Gly Ala 1 5 10 15	48
TCA GTG AAG CTG TCC TGC AAG GCT TCT GGC TAC ACC TTC ACC AGC TAC Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr 20 25 30	96
TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GGC CTT GAG TGG ATC Trp Met His Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile 35 40 45	144
GGA GAG ATT GAT CCT TCT GAT AGT TAT ACT AAC TAC AAT CAA AAG TTC Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe 50 55 60	192
AAG GGC AAG GCC ACA TTG ACT GTA GAC AAA TCC TCC AGC ACA GCC TAC Lys Gly Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr 65 70 75 80	240
ATG CAG CTC AGC AGC CTG ACA TCT GAG GAC TCT GCG GTC TAT TAC TGT Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys 85 90 95	288
GCA AGA TCG GAC TAC GGT AGT AGC CAC TTT GAC TAC TGG GGC CAA GGG Ala Arg Ser Asp Tyr Gly Ser Ser His Phe Asp Tyr Trp Gly Gln Gly 100 105 110	336
ACC ACG GTC ACC GTC TCC TCA Thr Thr Val Thr Val Ser Ser 115	357

Figure 8.**(A)**

GAC ATT GAG CTC ACC CAG TCT CCA ACC ACC ATG GCT GCA TCT CCC GGG Asp Ile Glu Leu Thr Gln Ser Pro Thr Thr Met Ala Ala Ser Pro Gly 1 5 10 15	48
GAG AAG ATC ACT ATC ACC TGC AGT GCC AGC TCA AGT ATA AGT TCC AAT Glu Lys Ile Thr Ile Thr Cys Ser Ala Ser Ser Ser Ile Ser Ser Asn 20 25 30	96
TAC TTG CAT TGG TAT CAG CAG AAG CCA GGA TTC TCC CCT AAA CTC TTG Tyr Leu His Trp Tyr Gln Gln Lys Pro Gly Phe Ser Pro Lys Leu Leu 35 40 45	144
ATT TAT AGG ACA TCC AAT CTG GCT TCT GGA GTC CCA GCT CGC TTC AGT Ile Tyr Arg Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser 50 55 60	192
GGC AGT GGG TCT GGG ACC TCT TAC TCT CTC ACA ATT GGC ACC ATG GAG Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Gly Thr Met Glu 65 70 75 80	240
GCT GAA GAT GTT GCC ACT TAC TAC TGC CAG CAG GGT AGT AGT ATA CCA Ala Glu Asp Val Ala Thr Tyr Tyr Cys Gln Gln Gly Ser Ser Ile Pro 85 90 95	288
CGC ACG TTC GGA GGG GGC ACC AAG CTG GAA ATC AAA CGG Arg Thr Phe Gly Gly Thr Lys Leu Glu Ile Lys Arg 100 105	327

(B)

GAG GTC AAG CTG CAG CAG TCA GGA CCT GAG CTG GTA AAG CCT GGG GCT Glu Val Lys Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala 1 5 10 15	48
TCA GTG AAG ATG TCC TGC AAG GCT TCT GGA TAC GCA TTC ATA AGT TTT Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Ala Phe Ile Ser Phe 20 25 30	96
GTT ATG CAC TGG GTG AAG CAG AAG CCT GGG CAG GGC CTT GAG TGG ATT Val Met His Trp Val Lys Gln Lys Pro Gly Gln Gly Leu Glu Trp Ile 35 40 45	144
GGA TTT ATT AAT CCT TAC AAT GAT GGT ACT AAG TAC AAT GAG AAG TTC Gly Phe Ile Asn Pro Tyr Asn Asp Gly Thr Lys Tyr Asn Glu Lys Phe 50 55 60	192
AAA GAC AAG GCC ACA CTG ACT TCA GAC AAA TCC TCC AGC ACA GCC TAC Lys Asp Lys Ala Thr Leu Thr Ser Asp Lys Ser Ser Ser Thr Ala Tyr 65 70 75 80	240
ATG GAG CTC AGC AGC CTG ACC TCT GAG GAC TCT GCG GTC TAT TAC TGT Met Glu Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys 85 90 95	288
GCA AGT GGG GAT TAC GAC AGG GCT ATG GAC TAC TGG GGC CAA GGG ACC Ala Ser Gly Asp Tyr Asp Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr 100 105 110	336
ACG GTC ACC GTC TCC TCA Thr Val Thr Val Ser Ser 115	354

INTERNATIONAL SEARCH REPORT

Intern'l Application No
PCT/EP 95/00978

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/13 C07K16/28 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>GENE, vol. 128,no. 1, 1993 AMSTERDAM, THE NETHERLANDS, pages 103-109, L. GARRARD ET AL. 'Selection of an anti-IGF-1 Fab from a Fab phage library created by mutagenesis of multiple CDR loops.' see abstract</p> <p>---</p>	1-10
X	<p>BIO/TECHNOLOGY, vol. 9,no. 12, December 1991 NEW YORK, NY, USA, pages 1373-1377, L. GARRARD ET AL. 'Fab assembly and enrichment in a monovalent phage display system.'</p> <p>see abstract</p> <p>---</p>	1-10

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
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- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
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- *&* document member of the same patent family

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Date of the actual completion of the international search 23 May 1995	Date of mailing of the international search report 07.06.95
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INTERNATIONAL SEARCH REPORT

Internal Application No
PCT/EP 95/00978

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NATURE, vol. 319, 16 January 1986 LONDON, GB, pages 230-234, T. YAMAMOTO ET AL. 'Similarity of protein encoded by the human c-erb-B-2 gene to epidermal growth factor receptor.' see abstract see figure 3 ---	1-10
A	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE USA, vol. 89, no. 10, 15 May 1992 WASHINGTON, DC, USA, pages 4285-4289, P. CARTER ET AL. 'Humanization of an anti-p185HER2 antibody for human cancer therapy.' see the whole document ---	1-10
A	NATURE, vol. 348, 6 December 1990 LONDON, GB, pages 552-554, J. MCCAFFERTY ET AL. 'Phage antibodies: filamentous phage displaying antibody variable domains.' see the whole document ---	1-10
A	NATURE, vol. 352, 15 August 1991 LONDON, GB, pages 624-627, T. CLACKSON ET AL. 'Making antibody fragments using phage display libraries.' cited in the application see abstract ---	1-10
A	JOURNAL OF MOLECULAR BIOLOGY, vol. 222, no. 3, 1991 LONDON, GB, pages 581-597, J. MARKS ET AL. 'By-passing immunization. Human antibodies from V-gene libraries displayed on phage.' cited in the application see abstract ---	1-10
A	PROTEIN ENGINEERING, vol. 4, no. 7, October 1991 OXFORD, GB, pages 773-783, C. KETTLEBOROUGH ET AL. 'Humanization of a mouse monoclonal antibody by CDR-grafting: the importance of framework residues on loop conformation.' cited in the application see the whole document ---	1-10
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INTERNATIONAL SEARCH REPORT

Internat Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>NUCLEIC ACIDS RESEARCH, vol. 19,no. 15, 11 August 1991 OXFORD, GB, pages 4133-4137, H. HOOGENBOOM ET AL. 'Multi-subunit proteins on the surface of filamentous phage: methodologies for displaying antibody (Fab) heavy and light chains.' cited in the application see the whole document</p> <p>---</p>	1-10
P,X	<p>EUROPEAN JOURNAL OF IMMUNOLOGY, vol. 24,no. 4, April 1994 WEINHEIM, GERMANY, pages 952-958, C. KETTLEBOROUGH ET AL. 'Isolation of tumor cell-specific single-chain Fv from immunized mice using phage-antibody libraries and the re-construction of whole antibodies from these antibody fragments.' cited in the application see the whole document</p> <p>-----</p>	1-10